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CHRONOLOGICAL LIST OF SESSIONS

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MONDAY

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202.5 Aspartate as a candidate transmitter in the Limulus neuromuscular preparation. S.G. Rane* & G.A. Wyse, Dept. of Zoology, University of Massachusetts, Amherst, MA 01003

Glutamate (GLU) is considered the best if not the only excitatory transmitter candidate for lobster, crayfish and locust neuromuscular junctions. Aspartate (ASP) is not generally considered to be a likely transmitter candidate although it is a weak agonist at these junctions. For this study both ASP and GLU were examined as excitatory transmitter candidates for the tibia flexor muscle of the chelicerate arthropd, Limulus polyphemus.

Bath application of ASP or GLU caused dose-dependent depolarizations of Limulus muscle fibers and contractions of the whole muscle. GLU had a lower threshold concentration than did ASP (0.01 vs. 0.1 mM) and GLU caused larger depolarizations at all concentrations tested (up to 3 mM). At or above 1 mM, however, ASP gave stronger, more prolonged contractions than did GLU. Both the excitatory postsynaptic potential (EPSP) and ASP and GLU depolarizations were associated with conductance increases in muscle fibers. ASP depolarizations were abolished in saline in which TRIS replaced Na^+ while GLU depolarizations were either slightly reduced or unaffected. ASP contractions were abolished but GLU contractions were augmented in saline in which sucrose replaced Na^+. The ionic basis of the Limulus EPSP could not be experimentally examined, but EPSPs in other arthropods are due mainly to a transmitter-dependent increase in Na^+ conductance.

High-performance liquid chromatography with fluorescence detection (OPA/ethanethiol derivatization) showed that motor axon stimulation caused increase in resting levels of ASP (110±40%), GLU (240±135%) and 8 other amino acids in fluid bathing the Limulus neuromuscular preparation (N=8). Pentobarbital (PB) at 0.5 mM reduced EPSPs and muscle contractions by 70-80%. PB also abolished stimulus-induced increases in GLU and 5 of 8 other amino acids, but it did not affect increases in ASP or the other 3 amino acids (N=7). Increases in all amino acids were abolished by zero-Ca^{++} saline (N=7). These results suggest that postsynaptic blockade of muscle activity by PB was sufficient to eliminate efflux from the preparation of GLU and 5 of 8 other amino acids; but that efflux of ASP and 3 other amino acids was dependent on motor axon stimulation alone. Of this latter group only ASP was physiologically active.

Based on its stimulus-induced release behavior and the ionic basis of its depolarization, ASP is a better candidate than GLU for the Limulus neuromuscular transmitter.

202.6 LIMULUS CARDIOEXCITATORY PEPTIDE. W. White,* W.H. Watson. Zoology Dept., UNH, Durham, NH 03824.

The heart of the horseshoe crab, Limulus polyphemus, is neurogenic. Compounds which modulate heart activity can act at a variety of sites. For instance, catecholamines influence the pacemaker cells, follower cells, and cardiac muscle (Watson & Augustine, Peptides 3, 485-492, 1982), while the peptide proctolin only affects cardiac muscle (Watson et al., J.E.B. 103, 55-73, 1983). We now report the isolation of another cardioactive peptide from the brain of the horseshoe crab. Its primary effect is to increase the heart rate by acting directly on the cardiac ganglion.

The initial separation of this peptide was carried out using gel filtration chromatography. Brains from 200 horseshoe crabs were boiled, homogenized, centrifuged, filtered, and applied to a Sephadex G-25 column. Fractions were dried, diluted with seawater, and assayed on Limulus hearts. Cardioexcitatory activity was present in fractions eluting just after the void volume, indicating a molecular weight of approximately 5000. A similar estimate of M.W. was obtained using a G-50 column.

The peptide was further purified using reversed phase HPLC. Active material eluted at a concentration of 38% acetonitrile on two successive HPLC runs, indicating it probably contains several hydrophobic amino acid residues. Incubation of partially purified fractions with either pronase or trypsin destroyed bioactivity, confirming that the active substance is a peptide.

The primary effect of this peptide is to cause a long-lasting increase in heart rate. Pulse application of 5 brain equivalents of the peptide caused a 100% increase in heart rate that gradually decreased to control levels during a 1 hr wash. Comparable effects on rate are observed when the peptide is applied to isolated cardiac ganglia. In addition to its chronotropic effects on the Limulus heart, this peptide causes relaxation of the Limulus gut (like FMRFamide) and moderate activation of the ventilatory central pattern generator in the ventral nerve cord. It also has FMRFamide-like actions on the Busycon radula protractor muscle. Thus, it may represent a 4th FMRFamide-like peptide in Limulus.

We would like to thank Jim Groome, Tom O'Donohue, and John Bishop for their assistance on many aspects of this study. This work was supported by NINCDS grant 19053-01.

202.7 5,7-DIHYDROXYTRYPTAMINE (5,7-diHT) EFFECTS ON AN IDENTIFIED SEROTONERGIC NEURON IN HELISOMA TRIVOLVIS. D. Gadotti, K.L. Lukowiak*, L.G. Bauce* and A.G.M. Bulloch. Dept. of Medical Physiology, University of Calgary, Calgary, Alberta., Canada T2N 4N1.

5-7-diHT is a toxic serotonin analogue used to deplete serotonin content, thought to act via axotomy of serotonergic pathways . We tested the feasibility of selectively axotomizing serotonergic neurons in the snail Helisoma trivolvis. The study focused on an identified neuron, C1, in the cerebral ganglion whose axon contributes the only serotonergic input to the buccal ganglion, the function of this input in feeding behavior having been defined.

Snails were injected with 5,7-diHT (1-5 mM initial blood concentration) and the animals dissected 2 to 15 days later. HPLC analysis for catecholamine and indolamine content in the ganglia showed a substantial and specific decrease (55-85%) of serotonin content in the buccal ganglia that represents the serotonin present in the axons and terminals of C1. In contrast, a minimal decrease of serotonin occurred in the cerebral ganglion where the serotonergic cell bodies are located; no changes occurred in control animals injected with carrier solution and contents of other indolamines and catecholamines were also unaffected.

Glyoxylic acid histofluorescence was employed to obtain a qualitative estimate of content in catechol- and indolamines; the histofluorescence was markedly decreased in the buccal ganglia of experimental animals. The morphology of C1 axons was studied by staining preparations with a fluorescein-conjugated serotonin antibody (Immunonuclear, Stillwater MN) which is more sensitive than glyoxylic acid. This method showed the axonal morphology of C1 in drug treated animals not to be different from controls. The axonal integrity of C1 was also investigated with intracellular injections of the dye Lucifer Yellow CH and there was no difference in the axonal branching pattern in serotonin-depleted snails versus controls. Electrophysiological studies are in progress.

In conclusion, our data show that 5,7-diHT is a specific tool as serotonin depletor, but, in our molluscan model, its action is not accomplished via axotomy. (Supported by Alberta Heritage Foundation for Medical Research, & MRC, Canada).

202.8 IDENTIFICATION OF SEROTONIN CONTAINING NEURONS IN GASTROPOD MOLLUSKS USING COBALT BACKFILL, FORMALDEHYDE-INDUCED FLUORESCENCE, AND ANTIBODY TECHNIQUES. R. D. Longley* and A. J. Longley. Pacific Sciences Institute and Friday Harbor Laboratories, Friday Harbor, WA 98250.

The axon pathway of the giant cerebral neuron (GCN) in the buccal ganglia of Tritonia diomedea was examined using cobalt backfills of the cerebrobuccal connectives, formaldehyde-induced fluorescence of freeze dried material, and anti-serotonin antibodies. GCN axons in the buccal ganglia were identifed in cobalt backfills by their unique branching pattern in the buccal nerves. The major branch of the GCN axon enters the ganglion ventrally from the posterior part of the connective and ascends laterally to the dorsal part of the neuropil where it then passes through the commissure and dorsal part of the contralateral ganglion neuropil. Cobalt backfills of both connectives in the same preparation showed the left and right GCN axons paralleling one another through the buccal commissure and the neuropil of the ganglia, usually within one axon diameter. Formaldehyde-induced fluorescence of the serotonergic GCN axons in the buccal nerves was consistent with the results of the cobalt technique. In the gastroesophageal ganglia fluorescence was limited to axons passing through the ganglia. Two bilaterally symmetric pairs of neurons, more fluorescent than the serotonergic axons, were located relative to other identified neurons in the buccal ganglia.

Results with anti-serotonin antibody using the method of Beltz and Kravitz (J. Neurosci. 3:585-602) were consistent with those of the methods above for the GCN axons. These axons, with their branching in the buccal ganglion neuropil, could be clearly identified in wholemounts. Small processes from these branches were associated with neuron somata and, in many cases, passed between the somata and the buccal ganglion sheath. In paraffin secions of the larger buccal ganglion neurons, serotonin immuno-reactive processes could be seen in invaginations of the axon hillock adjacent to the nucleus. Serotonin immuno-reactivity was not seen in neuron somata of the buccal and gastroesophageal ganglia or in peripheral neurons associated with the salivary duct.

Specificity of the antibody was tested on serial sections of Tritonia GCN somata. Antibody binding in the GCN was eliminated by preincubation with serotonin, but was unaffected by octopamine or BSA (each at 1 mg/ml). Additional tests on buccal ganglia in other species and on the dopamine containing neuron in Lymnaea RPdG indicated the specificity of the antibody for serotonin in molluscan neurons.

202.9 *MEASUREMENT AND CONTROL OF DOPAMINE AND SEROTONIN RELEASE FROM LIMAX GANGLIA IN VITRO.* S. J. Wieland, E. Jahn* and A. Gelperin. Dept. Anatomy, Hahnemann Univ., Philadelphia, PA 19102, Dept. Biology, Princeton Univ., Princeton, NJ 08544, and AT&T Bell Labs., Murray Hill, NJ 07974.

Dopamine and serotonin are present in the cerebral and buccal ganglia of the slug Limax maximus, structures which are required for the generation and modulation of the feeding motor program (FMP) in response to sensory stimuli. Addition of exogenous dopamine in vitro can trigger FMP while addition of serotonin in vitro modifies the intensity of FMP (Wieland and Gelperin, J. Neurosci. 3: 1735, 1983). We wish to examine the roles of endogenous dopamine and serotonin in the modulation of the feeding response.

The release of endogenous dopamine and serotonin from individual cerebral ganglion-buccal ganglion preparations in vitro was detected and measured by high performance liquid chromatography followed by electrochemical detection. The connective tissue sheath surrounding the ganglia appeared to bind dopamine but not serotonin in vitro, thus acting as a dopamine-selective "buffer." Basal release of dopamine (0.2-0.7 pmol/30 min) was detected from sheathed ganglia; no release was detected from desheathed ganglia. On the other hand, in response to 50 mM K^+, desheathed ganglia released larger amounts of dopamine (5 pmol/30 min) than sheathed ganglia (approx. 1.5 pmol/30 min). Basal release of serotonin was not detectable from either sheathed or desheathed ganglia over 30 min in vitro. The potassium-induced release of serotonin was not strongly affected by the presence or absence of the sheath, being approximately 2.5 pmol/30 min in 50 mM K^+. Release of both dopamine and serotonin was blocked in low Ca^{2+} in the presence of 5 mM Co^{2+}: dopamine release was reduced by 75%, serotonin release was reduced by more than 90%.

Following high K^+ treatment for 5 to 15 min spontaneous activity recorded from buccal nerve roots was suppressed for 15 to 30 min and the FMP response to lip stimulation was suppressed for at least 45 min. However, the ganglia could recover from several exposures to high K^+ indicating that this treatment did not permanently damage the in vitro system. This will allow studies of general as well as selective transmitter depletion in vitro. (Supported by NIH Grant MH-39160.)

202.10 GENETIC INFLUENCES ON NEUROTRANSMITTER CONTENT OF IDENTIFIED NEURONS OF LYMNAEA STAGNALIS. G. Audesirk, T. Audesirk, R. McCaman, J. Ono. Biol. Dept., Univ. of Colorado-Denver, 1100 14th St., Denver, CO 80202; Beckman Research Institute of The City of Hope, Duarte, CA 91010.

Variability in the behavior of an individual organism, while partially attributable to environmental variables, is also influenced by factors under direct genetic control, including neural connectivity and neural metabolism. For example, correlations have been reported between the genetic background of mice, their behavior in a passive-avoidance task, and their total brain content of specific transmitters (Donovick et al., 1981). The large, individually identifiable single neurons of the gastropod brain have been successfully subjected to microchemical transmitter analysis (McCaman et al., 1979, 1984). We have applied these techniques to identified neurons of two laboratory-raised populations of Lymnaea stagnalis, a self-fertilizing hermaphroditic freshwater gastropod. One group (wild-type) was the offspring of a genetically mixed population. The second (inbred) was a strain resulting from six generations of inbreeding; each generation produced by self-fertilization of a single individual from the previous generation.

Neurotransmitter content was measured in two identified giant neurons in inbred and wild-type populations. The paired serotonergic cerebral giant neurons (LC1 and RC1) have significantly higher transmitter levels and less variability in inbred animals than in wild-type animals. The transmitter content of the unpaired dopaminergic right pedal giant neuron (RPeD1) does not differ between inbred and wild-type animals in either level or variability. It is proposed that serotonin content of the cerebral giant neurons is under partial genetic control, and that animals of the wild-type population possess a number of different alleles for the genes influencing serotonin levels. The wild-type population is probably already isogenic for genes influencing dopamine content in the right pedal giant neuron.

The finding that inbreeding results in greater homogeneity of transmitter content should facilitate comparison of transmitter content in groups of animals exposed to differing treatments in vivo.

202.11 Release Of ^{3}H-Glycine From Aplysia Neuron R14 Is Dependent On Both Frequency Of Firing And Duration Of Action Potentials. A.R. Rittenhouse & C.H. Price. Department of Biology, Boston University, Boston, MA 02215.

Identified neuron R14, located on the dorsal surface of the parietovisceral ganglion (PVG), uses free glycine as a neuromodulator to potentiate contraction of vascular smooth muscle. Previously, we showed that electrical stimulation of R14 axons preloaded with ^{3}H-glycine (^{3}H-G) and chemical stimulation of its soma both resulted in increases in ^{3}H-G released from R14 terminals in the anterior aorta (Rittenhouse & Price, Neurosci. Abstr., 9: 301, 1983). Electrophysiological tracing of R14 demonstrated its axons are present in the genital and pericardial nerves and terminate in the sheath of the digestive gland (DGS). In this study, we report that increased duration or frequency of firing of R14, preloaded with ^{3}H-G, elicit parallel increases in ^{3}H-G release from R14 terminals in the DGS.

The PVG, nerves, and DGS were dissected out and pinned such that the DGS occupied one chamber and the PVG another. The genital and pericardial nerves were passed through a water-tight barrier, dividing the two chambers. The PVG incubated in ^{3}H-G (50 or 100 µCi/ml) for 16-24 hr and the DGS was superfused with seawater medium. The incubation period allowed time for R14 to take up the ^{3}H-G, axonally transport it, and load up terminal regions in the DGS. Label was removed from the PVG chamber and both compartments were rinsed repeatedly to wash off extracellular ^{3}H-G. A sampling schedule was established so that the medium bathing the DGS was collected every 5 min for scintillation counting. Electrical activity in R14 was monitored with an intracellular electrode.

Bath application of 5 mM histidine onto R14 soma caused an immediate, 3-fold increase in firing rate (0.4-1.3 Hz) & a concomitant doubling of AP duration. The amount of ^{3}H-G collected from the DGS chamber rose 240% over control periods (N = 4), but returned to baseline within one sampling period. Application of 25 mM TEA doubled the duration of R14's spike, but did not alter its frequency. Lengthening only the duration of the spike resulted in a 150% increase in ^{3}H-G levels above control levels; a return to prestimulation levels occured within 5 min after TEA removal (N = 5). Thus, increasing both frequency and duration of R14 firing by histidine elicits 60% greater release of ^{3}H-G than release induced by duration alone with 25 mM TEA.

202.12 PEPTIDERGIC MODULATION OF A NEUROMUSCULAR JUNCTION IN THE MOLLUSC APLYSIA. J.E. Richmond*, A.G.M. Bulloch and K.L. Lukowiak* (Sponsor: A.D. Murphy). Dept. Physiology, University of Calgary, Calgary, Alberta., Canada T2N 4N1.

A variety of recent work has demonstrated peptidergic modulation of neurotransmission in the molluscan nervous system. For instance, the tetrapeptide FMRFamide potentiates the gill withdrawal reflex and gut contractions in Aplysia, whereas both of these are inhibited by arginine vasotocin (AVT). The purpose of the present study was to examine the cellular basis of the action of these peptides by utilizing a muscle preparation amenable to intracellular recording.

The preparation consisted of a buccal muscle of Aplysia with its attached nerve trunk. This was either pinned out at its insertion points for intracellular recording, or pinned at one insertion point and attached to an isotonic tension transducer. Single twitches were evoked by electrical shocks of the nerve trunk at once a minute.

The amplitude of stimulated contractions of the buccal muscle was markedly potentiated by FMRFamide. At concentrations of 10^{-6}M, twitch size was increased by 4 to 6 fold, with no change in baseline tension. This potentiation of contraction was rapidly reversible, and was dose-dependent, being measurable at concentrations as low at 10^{-12}M. In contrast, AVT inhibited these stimulated contractions and relaxed baseline tension, but was ineffective at concentrations less than 10^{-6}M.

The FMRFamide potentiated muscle twitches exhibited a decrease of rise time as well as the increase of amplitude. Although the amplitude of sub-maximal contractions could also be increased by increasing stimulus strength, such an increase is accompanied by an increase of rise time. It is unlikely, therefore, that FMRFamide acts upon inactive neuromuscular junctions. Peptides were also applied to the muscle during intracellular recording from individual muscle fibres. The amplitude of the evoked excitatory junction potential (EJP) was increased when the preparation was perfused with FMRFamide at concentrations of 10^{-9}-10^{-6}M, this also being accompanied by a decreased rise time.

In conclusion, FMRFamide and AVT modulate the neuromuscular junctions of Aplysia buccal muscle. The pre- and post-synaptic action of these peptides is currently under investigation. (Supported by MRC, Canada, & Alberta Heritage Foundation for Medical Research.

202.13 EVIDENCE FOR OPIOID MECHANISM IN APLYSIA CEREBRAL GANGLIA, M. K. Leung, A. F. Hall*, G. B. Stefano, A. Chapman* and D. O. Carpenter. Depts. of Chemistry and Biological Sciences, SUNY/Old Westbury, Old Westbury, NY 11568 and NYS Dept. of Health, Albany, NY 12201.

Leu-enkephalin was applied by pressure injection onto A and B cells of Aplysia cerebral ganglia. Changes were recorded by voltage-clamp and current-clamp. The results showed increases in Na^+, K^+ and Cl^- conductance. Late depolarization responses appeared to associate with a conductance decrease. Receptor desensitization responses appeared to associate with membrane conductance increases. However, these responses were not abolished by high concentration of noxolane. Thus, a delta receptor or a novel opioid receptor may be involved. Acid extract from the cerebral ganglia was analyzed by HPLC with a reverse-phase column using 10mM NH_4 acetate pH 4.0 and a gradient of 5-25% 2-propanol in 30 min. The results showed the presence of peptide peak with R_t same as Met-enkephalin. The peptide from this peak was purified by HPLC under isocratic condition. Displacement analysis showed the isolated peptide displaced ^{3}H-D-Ala^2-Met^5-enkephalinamide in a similar manner as authentic Met-enkephalin. These results suggested the presence of any opioid mechanism in Aplysia cerebral ganglia.
(Supported by NIH Grants MBRS RR08180 and MH17138)

202.14 IMMUNOCYTOCHEMICAL MAPPING OF THE NEURAL CONTROL SYSTEM FOR FEEDING IN LIMAX MAXIMUS. I. Cooke and A. Gelperin. Molecular Biophysics Research Dept., AT&T Bell Labs, Murray Hill, NJ 07974 and Dept. Biology, Princeton University, Princeton, NJ 08544.

The terrestrial slug Limax maximus shows rapid and reliable conditioning of both aversive and appetitive responses to food odors (reviewed in Gelperin, 1983). One-trial food aversion learning is obtained when novel, very attractive food odors are paired with noxious or toxic stimuli. The neural substrate of food ingestion, termed feeding motor program, can be studied in the isolated cerebral and buccal ganglia. These ganglia also show one form of food aversion learning. The basic pattern of feeding motor program can be generated by the buccal ganglia alone, however, normally the generation of feeding motor program involves interaction between cerebral and buccal ganglia. We are seeking to identify neurons in the cerebral and buccal ganglia that may be involved in the control and modulation of feeding behaviour as part of a search for the locus and mechanism of food aversion learning. Using the whole mount techniques of Beltz and Kravitz (J. Neurosci. 3: 585-602, 1983) we have demonstrated the presence of FMRF amide-like immunoreactivity (FLI) in the cerebral and buccal ganglia of Limax. Two clusters of small somata exhibiting FLI were found in each buccal ganglion. Other small somata with FLI were scattered through the buccal and cerebral ganglia. In addition, cell B1, a large identified buccal neuron known to innervate a variety of targets also showed FLI. Nerve fibres with FLI were observed in the buccal nerve roots, the cerebrobuccal connectives and some cerebral nerve roots. The outer sheath surrounding the cerebral ganglion also exhibited strong FLI. Experiments to determine the effect of FMRF-amide on the feeding system of Limax are in progress.

Preliminary experiments have indicated that α-aminobutyric acid (GABA) exerts a powerful inhibitory effect on the feeding system of Limax. We have found GABA-like immunoreactive nerve fibres in the CNS and are proceeding to localize the somata and terminal projections of these neurons.

Gelperin, A. 1983. Neuroethological studies of associative learning in feeding control systems. In: F. Huber & H. Markl, eds. Neuroethology and Behavioral Physiology, Springer-Verlag, Berlin, pp. 189-205.
(Supported by NIH Grant MH 39160.)

202.15 IDENTIFICATION OF FMRF-AMIDE IMMUNOREACTIVE NEURONS IN THE ABDOMINAL GANGLION OF APLYSIA. R. O. Brown†, A. I. Basbaum†§, and E. Mayeri†#. Depts. of †Physiology and §Anatomy, University of California, San Francisco, CA 94143 and #Dept. of Basic Sciences, California College of Podiatric Medicine, San Francisco, CA 94115.

We are studying the possibility that the molluscan cardioexcitatory peptide Phe-Met-Arg-Phe-amide (FMRFa) functions as a neurotransmitter in the abdominal ganglion of Aplysia californica. Previous studies have shown that FMRFa has widespread excitatory and inhibitory activities on abdominal ganglion neurons (Stone et al., Soc. Nsci. Abstr. 7:636), and that authentic FMRFa can be purified from abdominal ganglion extracts (B. Rothman et al., in prep.). In this study we used physiological and fluorescence-immunocytochemical techniques to identify neurons containing immunoreactive- (IR-) FMRFa.

Abdominal ganglia from 400-800 gram animals were fixed by perfusion with 4% paraformaldehyde. FMRFa antiserum, obtained from Dr. Eckard Weber, was used at 1:4000. Individual cells were identified electrophysiologically and filled with Lucifer Yellow by pressure injection.

The abdominal ganglion contained large amounts of IR-FMRFa. Preabsorption of the antiserum with 100 μM FMRFa eliminated the labelling. The neuropil was densely packed with IR-FMRFa fibers. Labelled fibers with beaded varicosities were also seen throughout the sheath overlying the ganglion; some fibers appeared to envelop certain neuronal somata.

A large number of cell bodies contained IR-FMRFa with varying intensities of labelling. Positively identified (Lucifer-filled) neurons labelling for FMRFa were L_2, L_3, L_4, L_5, L_6, R_2 and a previously unidentified cluster of 7-10 medium-sized white cells on the ventral surface of the right lower quadrant. Two other large cells, tentatively identified as L_{12} and L_{13}, were immunoreactive for FMRFa. IR-FMRFa was also seen in numerous small cells, often in clusters, throughout the ganglion. L_1, L_7, L_{10}, L_{11}, R_{3-13}, R_{14}, R_{15}, and the bag cells did not label for FMRFa.

The giant cholinergic neurons, LP_1 in the left pleural ganglion and R_2, both contain IR-FMRFa; this suggests that these two cells may use both acetylcholine and an FMRFa-like peptide as neurotransmitters.
Supported by NIH Grants NS10246, NS14627, and NS16033.

202.16 ATRIAL GLAND CELLS PRODUCE AND RELEASE AN IDENTIFIED FAMILY OF EGG-LAYING PEPTIDES IN APLYSIA. G.T. Nagle, S.D. Painter and J.E. Blankenship. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550

The atrial gland of Aplysia californica is an exocrine organ in the large hermaphroditic duct of the reproductive tract that produces peptides that can induce egg laying when injected into another animal. To define the major biosynthetic products, atrial glands were labelled in vitro by exposure to a mixture of ^{3}H-amino acids for 18h, extracted in acid in the presence of peptidase inhibitors, purified by Sephadex G-50 gel filtration, and the 2-10kD peptides isofocused. Scintillation counting of sequential 1mm gel slices reveals a complex but highly reproducible pattern of radiolabelled peptide peaks. A surprisingly large number of these peaks induce apparently normal egg laying when eluted and injected into intact animals. Each active peak was further screened in a bag cell discharge neuroassay (for peptide A- and B-like activity) and by injection into bag cell-less Aplysia (for egg-laying hormone (ELH)-like activity). The active peaks were classified into one of three categories: those containing (1) ELH-like activity, (2) peptide A- and B-like activity, or (3) egg-releasing hormone (ERH)-like activity. Peaks with ERH-like activity are active in both assays. Each peak was assessed for homogeneity on an SDS-PAGE system that separates low molecular weight peptides with high resolution.

Interestingly, 18h-labelled atrial glands rapidly and spontaneously release their peptide products in vitro, whether chased in normal or low Ca^{++}-high Mg^{++} seawater containing peptidase inhibitors. When the releasate is analyzed following gel filtration and isofocusing, the pattern of released 2-10kD radiolabelled peptides is very similar to that seen in freshly labelled 18h glands. Furthermore, the releasate, like the labelled glands, contains peaks with ELH-like, peptide A- and B-like, as well as ERH-like activities. It is conceivable, however, that the rapid, quantitative peptide release observed in vitro does not accurately reflect the normal functioning of the atrial gland in intact Aplysia; we are investigating whether this spontaneous release is the result of removing an otherwise quiescent gland from some form of tonic inhibition in vivo.
Supported by NIH NS 07025(GTN), NS 07010(SDP), NS 11255 and NSF PCM 82 15185(JEB).

202.17 SPECIES SPECIFICITY OF MONOCLONAL ANTIBODIES PRODUCED AGAINST BIOLOGICALLY ACTIVE PEPTIDES FROM THE ATRIAL GLAND OF APLYSIA CALIFORNICA. S.D. Painter, V.K. Kalman*, G.T. Nagle and J.E. Blankenship. Marine Biomedical Institute, Univ. of Tex. Med. Br., Galveston, TX. 77550

The atrial gland of the sea hare Aplysia californica is an exocrine organ in the large hermaphroditic duct of the reproductive tract. Aqueous extracts of this organ induce egg laying when injected into A. californica, A. dactylomela or A. brasiliana. Aqueous extracts of the A. brasiliana and A. dactylomela ducts also induce egg laying when injected into A. californica, suggesting that the active factors must be similar in the three species. We have localized the egg-laying activity in the A. dactylomela and A. brasiliana ducts by dissection and bioassay: activity is restricted to the atrial gland in A. dactylomela and to a narrow glandular region bordering the red hemiduct in A. brasiliana. Both A. dactylomela and A. brasiliana have a prominent pea-shaped gland in the anterior large hermaphroditic duct; it does not contain egg-laying activity in either species.

The active areas of the A. brasiliana and A. dactylomela ducts are morphologically very similar to the atrial gland of A. californica, although the area is never as elaborated in A. brasiliana as in the other two species. Each area is a stratified epithelium composed of non-ciliated columnar epithelial cells overlaid by ciliated capping cells, which cover most of the lumenal surface. The columnar cells have basal nuclei and large (1-2μm in diameter) secretory granules. Some cells have large blue (H & E) or pink (cresyl violet) pools filling the apical portion of the cell. A third type of columnar cell is recognizable in the A. dactylomela atrial gland (cresyl violet): these cells have distinctive deep purple granules and appear to be actively secreting into the lumen of the duct.

We have produced polyclonal and monoclonal antibodies against the biologically active atrial gland peptides of A. californica. The mouse polyclonal antibodies selectively stain the large secretory granules of the atrial gland columnar epithelial cells, but do not stain the atrial gland capping cells nor other parts of the duct. A monoclonal line, FW-6G8, retains these staining characteristics in A. californica. When tested on A. brasiliana and A. dactylomela large hermaphroditic ducts, the polyclonal serum stains large secretory granules in the columnar epithelial cells of the active regions. The monoclonal line, in contrast, does not stain any structures in either duct. Supported by NIH NS 07010(SDP), NS 07025(GTN), NS 11255 and NSF PCM 82 15185(JEB).

NEUROTRANSMITTERS, MODULATORS: COEXISTENCE OF TRANSMITTERS

203.1 COEXISTENCE OF PROCTOLIN WITH TRH AND 5-HT IN THE RAT CNS. V.R. Holets[1], T. Hökfelt[1]*, J. Ude[2]*, M. Eckert[2] * and S. Hansen[3]*. [1]Department of Histology, Karolinska Institutet, 104 01 Stockholm, Sweden; [2]Dereich Tierphysiologie der Sektion Biologie, Friedrich-Schiller-Universität, Jena, DDR; and [3]Department of Psychology, Göteborgs Universitet, 400 20 Göteborg, Sweden.

The pentapeptide proctolin has been localized in the CNS of many arthropods, as well as in the leech, lobster and crayfish CNS. Proctolin has been shown to be co-contained with a monoamine in cells in the grasshopper and cricket. Using the indirect immunofluorescence technique, the distribution of proctolin-like immunoreactivity (PLI) in the rat CNS was investigated using a specific polyclonal rabbit antiserum raised against proctolin. Normal and colchicine-treated rats (120 μg/20 μl saline; lateral ventricle) were perfusion fixed with a modified Zamboni fixative. Serial 5-14 μm sections were cut through the hypothalamus, raphe nucleus and all levels of the spinal cord. Adjacent sections were used for the localization of TRH-like and 5-HT-like immunoreactivity. Specificity of the proctolin antiserum was determined by RIA and by preabsorbing the antiserum with 10-100 μg/ml of proctolin or TRH. No decrease in the intensity of staining was observed with the addition of TRH, but 10 μg/ml of proctolin completely absorbed all the staining observed with the proctolin antiserum. PLI was localized in cell bodies in the paraventricular nucleus of the hypothalamus (PVN), nucleus raphe obscurus (NRO) and nucleus raphe pallidus (NRP). The PLI was found to coexist with TRH in a select population of neurons in the PVN, and to coexist with TRH and 5-HT in the NRO and NRP. The majority of neurons which contained PLI also contained TRH in the PVN, and TRH and 5-HT in the NRP and NRO, but not all TRH or 5-HT immunoreactive neurons contained PLI. Fibers containing PLI were localized to the PVN, the median eminence and the nucleus solitarius. In the spinal cord, PLI was found in fibers in the lateral horn of the thoracic level, surrounding the central canal and in the ventral horn at all levels of the spinal cord, following the distribution of TRH fibers in the spinal cord. No PLI was observed in the dorsal horn at any spinal cord level. The role of proctolin coexistent with TRH and 5-HT in the spinal cord and its role in sexual behavior are presently being investigated. Supported by a Fogarty Fellowship from the SMRC (V.R.H.) and SMRC Grant 04X-2887 (T.H.).

203.2 CHOLECYSTOKININ (CCK) IN RAT PITUITARY NEUROINTERMEDIATE LOBE (NIL) DURING THE ESTROUS CYCLE. S. Goldman*, O. Van Reeth*, S. Schiffmann*, F. Lotstra* and J.J. Vanderhaeghen. Neuropath. Neuropept. Res. Lab., Hôpital Erasme, Université Libre de Bruxelles, Fondation Médicale Reine Elisabeth. 1 J.J. Crocq Ave, B-1020, Brussels, Belgium.

Coexistence of CCK and oxytocin has been shown in several nerve cell bodies of rat and bovine paraventricular and supraoptic nuclei. (Vanderhaeghen et al., Proc. Natl. Acad. Sci. USA, 77, 1190-1194, 1980; Cell Tiss. Res., 221, 227-231, 1981). Lower CCK content in the NIL has been demonstrated in some conditions associated with oxytocin secretion (Beinfeld et al, Nature, 288, 376-378, 1980). Compared with the male, a lower NIL CCK content with a wider distribution has been demonstrated in the female rat (Deschepper et al., Life Sci., 32, 2571-2577, 1983). We here report a significant fluctuation of CCK in the NIL during the various stages of the estrous cycle. Vaginal smears were taken in 40 Wistar female rats to determine the estrous stage (rats with 5 consecutive 4-day estrus cycle were used). The rats were sacrified by decapitation, the NIL was immediately dissected and then homogenized and boiled in distilled water. After centrifugation, supernatants were used for determination of CCK content by RIA.

Stage of estrous cycle	CCK content in pmol/g wet weight ± s.e.m.	n	P <
Proestrus and estrus	602 ± 21	17	
Metestrus and diestrus	494 ± 30	14	0.005*
			0.01+
Proestrus	589 ± 36	8	
Estrus	614 ± 26	9	
Metestrus	512 ± 27	6	0.05*
Diestrus	480 ± 49	8	0.05*
			0.05+

*P value when compared to proestrus and estrus. +P value when compared to estrus (U-Mann-Withney test).

The variation of neurohypophyseal CCK content reported here during the estrous cycle is similar to the one already reported for oxytocin. Our results point to a common influence of sexual steroids on oxytocin and CCK in the hypothalamo-neurohypophyseal axis. Supported by FRSM(3.451.82-85), FNRS, FMRE, ANAH and Fondation Erasme 1983-84.

203.3 PUTATIVE ROLE OF γ_1-MSH AT CNS LEVEL IN RAT. W. Fratta, H.-Y.T. Yang and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

Processing of proopiomelanocortin (POMC) leads to the formation of three distinct families of regulatory neuropeptides, namely endorphins, ACTH, α- and γ-MSH peptides, in pituitary and brain structures. Thus, POMC containing neurons could offer an interesting model of, at least, triple coexistence of neuromodulators. Among the wide spectrum of possible neurophysiological roles proposed for the ACTH-MSH like peptides, several lines of independent evidence have suggested that these peptides could act as endogenous opiate antagonists. Very little is known on the biological properties and possible physiological role of the γ-MSH peptide family. Here, we show that γ_1-MSH, the endogenous amidated product of γ-MSH, has a biological profile which resemble that of an opiate agonist. In fact, we found that γ_1-MSH inhibits the electrically induced contractions in the guinea pig ileum myenteric plexus-longitudinal muscle ($IC_{50}=10^{-5}M$). This effect was reversed by naloxone in a dose dependent manner ($IC_{50}=10^{-7}M$). Furthermore γ_1-MSH potentiated the depressant effect of either β-endorphin or leu-enkephalin in this system. Either $ACTH_{1-24}$ or α-MSH or $ACTH_{4-10}$ failed to have opiate agonist action up to a concentration of $10^{-5}M$. However, $ACTH_{1-24}$ added in bath either before or after γ_1-MSH inhibited in dose related manner the depressant effect of γ_1-MSH (complete inhibition at equimolar concentrations). α-MSH and $ACTH_{4-10}$ displayed the same antagonistic activity but with less potency, indicating that the amino acidic sequence 4-10 of the ACTH which is contained also in α- and β-MSH seems to be essential for this action as well as for most of the central effects of ACTH. In rat brain membranes γ_1-MSH specifically displaced ^{3}H-dihydromorphine, ^{3}H-ethylketocyclazocine and ^{3}H-D-Ala^2-D-Leu^5 enkephalin binding with IC_{50} ranging from 10^{-7} to $5x10^{-7}M$. Intracerebroventricular injections of 5 or 10 μg of γ_1-MSH hardly affected the tail-flick response in rats and produced barrel rotations as reported also after dynorphin injections. Furthermore, the most characteristic central effects of ACTH, namely stretching-yawning syndrome, were antagonized by the concomitant injection of γ_1-MSH. On the basis of these results the possibility emerges that γ_1-MSH may function as antagonist of nonhormonal central actions of ACTH and as a synergist of the opioid-like action of endorphins. All these data taken together suggest that the β-endorphin antagonist action of ACTH may be related to its interaction with γ_1-MSH, thus the coexisting three neuropeptides could interact with each other, functionally.

203.4 VIP DECREASES ACETYL CHOLINE TURNOVER IN SALIVARY GLAND. C. Eva* and J. L. Meek (SPON: S. Stine). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington D.C. 20032.

Acetylcholine (ACh) and vasoactive intestinal peptide (VIP) probably coexist in cholinergic neurons of salivary glands. VIP like immunoreactive nerve fibers occur in cholinergic fibers around blood vessels and secretory elements in this tissue. In cat salivary glands, cholinergic drugs regulate both ACh and VIP release from parasympathetic nerve endings, presumably via a feedback loop. In this work, we investigated whether VIP could modulate the metabolism of ACh in mouse submandibular gland cholinergic neurons using the ACh turnover rate as a parameter. The TR_{ACh} was measured by ^{3}H-choline incorporation into ACh during constant rate infusion (1 μCi/min, 80 μCi/mmol). Mice were microwaved after different infusion times and tissues were prepared by precipitation of amines with Reineckate salt. Choline and ACh were separated by reverse phase HPLC, and detected electrochemically using an enzyme loaded post-column reactor. Fractions collected from the HPLC eluate were used for the determination of Ch and ACh specific activities. We calculated that the TR_{ACh} was about 3.1 mmol/mg prot/hr. Pilocarpine, a muscarinic agonist, decreased the TR_{ACh} about 3 folds, while atropine, a muscarinic antagonist caused a large increase in turnover. Turnover therefore appears to be regulated by a feedback mechanism triggered by occupancy of postsynaptic receptors. VIP, infused intravenously (30 μg/kg/min) decreased the TR_{ACh}. These results show that VIP, a putative cotransmitter with ACh in the salivary gland, is able to control the ACh metabolism in cholinergic neurons. This suggests that by changing postsynaptic receptor function, VIP participates in the feedback regulation of ACh metabolism.

203.5 PEPTIDE-LIKE IMMUNOREACTIVITY COEXISTS WITH GLUTAMIC ACID DECARBOXYLASE IMMUNOREACTIVITY IN NEURONS OF CAT AND MONKEY CEREBRAL CORTEX. S.H.C. Hendry, J. DeFelipe* and E.G. Jones, Washington University School of Medicine, St. Louis, MO 63110 and University of California, Irvine, CA 92717.

Neurons in the cat and monkey (Macaca fascicularis) cerebral cortex (sensory-motor, parietal and visual areas) displaying immunoreactivity for somatostatin (SRIF), neuropeptide Y (NPY), cholecystokinin octapeptide (CCK) and glutamic acid decarboxylase (GAD) were examined light and electron microscopically. Neurons stained for each of the four substances are non-pyramidal cells. Using methods for sequential or simultaneous localization of two antigens, we determined: (1) All neurons displaying CCK-like, SRIF-like or NPY-like immunoreactivity in cat cortex and the vast majority in monkey cortex are also GAD-positive; (2) At least one-quarter of the total population of SRIF- and NPY-positive cells are immunoreactive for both peptides; (3) No CCK-positive cell was found to be SRIF- or NPY-positive; (4) The number of GAD-positive neurons displaying immunoreactivity for CCK,SRIF or NPY is small in comparison with those that are GAD-positive alone. Most CCK-positive axon terminals form symmetric synapses onto cell bodies and proximal dendrites of unlabeled cells mainly in the superficial layers while most SRIF- or NPY-positive axon terminals form symmetric synapses onto small dendrites or dendritic spines. GAD-positive terminals form symmetric synapses at all of these and at many other sites, including the cell bodies and proximal dendrites of cells in the deep layers and the initial segments of pyramidal cell axons. This implies that large basket cells and chandelier cells, though GAD-positive, are not immunoreactive for any of the three peptides. We conclude that GAD-positive neurons in cat and monkey cortex include at least three separate populations - one that is also CCK-positive and makes synapses on cell bodies and proximal dendrites of some neurons, a second that is also SRIF- and NPY-positive and makes synapses on small dendrites and dendritic spines, and a third in which CCK-, SRIF- and NPY-like immunoreactivity are not detectable and that make synapses on many different neuronal elements.

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203.6 CHOLECYSTOKININ ANTAGONISTS BLOCK THE POTENTIATION OF DOPAMINE-INDUCED HYPERLOCOMOTION BY CHOLECYSTOKININ IN THE NUCLEUS ACCUMBENS. J.A. Stivers* and J.N. Crawley. Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20205.

Cholecystokinin co-exists with dopamine in mesolimbic neurons in rat brain. When injected directly into the nucleus accumbens, CCK_8 potentiated dopamine-induced hyperlocomotion and apomorphine-induced stereotypy, over a dose range of 20 pg - 200 ng. Unsulfated CCK_8 was ineffective over a wide dose range. CCK administered alone had no effect on locomotion or stereotypy, suggesting that CCK is primarily a modulator of dopamine-mediated behaviors in the mesolimbic system. CCK did not potentiate apomorphine-induced stereotypy when injected into the caudate nucleus, where CCK and dopamine are located in different neurons, suggesting that the potentiation effect is specific to the neuromodulator-neurotransmitter co-existence phenomenon.

To test the pharmacological specificity of the CCK-induced potentiation of dopamine-induced hyperlocomotion in the nucleus accumbens, specific antagonists of CCK were injected into the nucleus accumbens prior to administration of saline, dopamine 20 µg/side + saline, or dopamine 20 µg/side + sulfated CCK_8 200 pg/side. Proglumide, 20 µg/side, had no effect on locomotion when administered alone, or in conjunction with dopamine, but effectively blocked the potentiation by CCK when administered in conjunction with dopamine + CCK. Benzotript, 10 µg/side, also effectively blocked the potentiation by CCK. Rabbit antibodies raised against sulfated CCK_8 (gift of Dr. M.C. Beinfeld, Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO), similarly blocked the CCK-induced potentiation of dopamine-induced hyperlocomotion without affecting locomotion when given alone or prior to dopamine administration. Preimmune serum was not active in blocking these behavioral effects of CCK.

These data show that the modulatory effects of CCK on dopamine-mediated behavior, at a site of co-existence in the rat brain, can be blocked by specific antagonists of the CCK receptor.

203.7 CHOLECYSTOKININ SELF-INJECTION IN THE NUCLEUS ACCUMBENS AND BLOCK WITH PROGLUMIDE. B.G. Hoebel and E. Aulisi. Dept. Psychology, Princeton Univ., Princeton, NJ 08544

Eight rats with cannulas in the nucleus accumbens responded on the appropriate one of two levers for 65±15 nl, unilateral injections of 0.4 ug/nl sulfated cholecystokinin (CCK) at a mean rate of 21 presses per hour during 4-hr sessions. This rate approximately doubled when the concentration was halved or when 0.8 ng/nl of the CCK blocker, proglumide, was added to the self-injection mixture. Conversely, doubling the CCK concentration halved the response rate. Thus the animals changed their response rate to compensate for a CCK blocker or for changes in concentration. Proglumide added to the CCK at the dose tested had the same effect as halving the concentration of CCK.

This result demonstrates self-regulation of a non-opiate peptide in a local brain region. It is similar to self-injection of amphetamine and block by the dopamine antgonist, flupenthixol, in this same region (Hoebel, B.G., et al. Psychopharmacol., 81:151, 1983; Aulisi, E. & Hoebel, B.G., Soc. Neurosci. Abstr. #36.7, p. 121, 1983). Given that CCK and dopamine have been colocalized in the nucleus accumbens, it would appear that the two cotransmitters have similar functions in the reinforcement of behavior. The CCK antagonist, proglumide, may function in a manner analogous to a neuroleptic. (Supported by USPHS Grant MH-35740 and Squibb Inst. for Med. Res.)

203.8 GALANIN IMMUNOREACTIVE NEURONS IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM. T. Melander*, T. Hökfelt*, Å. Rökaeus*, K. Tatemoto* and V. Mutt* (SPON: S. Whittemore). Departments of Histology, Pharmacology and Biochemistry, Karolinska Institutet, Stockholm, Sweden.

Galanin (GAL) was isolated from pig small intestine and characterized as a 29 amino acid peptide (Tatemoto, K. et. al., FEBS Letters, 164: 124-128, 1983). Using an antiserum raised against pig GAL conjugated to bovine serum albumin, GAL immunoreactive (IR) neuronal structures have been mapped in detail in the central and peripheral nervous system (CNS and PNS) of several species. In the spinal cord small GAL positive cells were detected in laminae I and II of the dorsal horn together with a moderately dense fiber network. In the medulla oblongata GAL-IR cell bodies were seen in the caudal spinal trigeminal nucleus, also here with GAL-positive fiber networks. An extensive system of GAL-IR cell bodies and densely aggregated fibers were observed in the dorsal vagal complex. In the ventrolateral area of the medulla large positive perikarya could be detected together with a moderately dense fiber network. In the pons and mesencephalon a very large proportion of the neurons of the locus coeruleus were GAL-positive. A medium dense to dense GAL-IR innervation of the periaqueductal central grey could be noted. In the diencephalon GAL-positive cell bodies could be seen in the anterodorsal and periventricular thalamic nuclei, lateral to the mammillary recess, in the arcuate nucleus, anterior to and in the dorsomedial hypothalamic nucleus, in the medial forebrain bundle area, in the medial preoptic area and in the anterior periventricular region of the hypothalamus. Neurons in the paraventricular, supraoptic and caudal magnocellular nuclei were GAL-positive. Wide-spread fiber systems, with the highest concentrations in the dorsal and periventricular aspects of the hypothalamus and in the median eminence, were detected. In the telencephalon several large populations of GAL-IR somata were located in and around the nucleus of the diagonal band and in the central amygdaloid nucleus. In the PNS GAL-positive structures were mainly found in the gastro-intestinal tract. However, a large portion of the chromaffin cells of the adrenal medulla stained for a GAL-like substance. In some cases central GAL-positive neurons contained one or several other peptides or a marker for a classical transmitter, indicating that this GAL-like peptide is involved in coexistence situations at several levels of the CNS. (SMRC 04X-2887)

203.9 EFFECTS OF A PEPTIDE FROM APLYSIA NEURONS R3-R14 ON POTENTIAL TARGETS. H. Yamagishi*, C.Y. Lin* and D.J. McAdoo. (SPON: D.L. Trevino). Marine Biomedical Institute, Univ. of Texas Med. Br., Galveston, TX 77550.

Identified Aplysia neurons R3-R14 have many characteristics of neurosecretory neurons (Coggeshall et al., 1966), including the presence of characteristic peptides (Nambu et al., 1983 and references therein). We have isolated several R3-R14 peptides by dissecting out R3-R14 cell bodies, extracting them with 0.5 N formic acid and resolving the components of the supernatant by high pressure liquid chromatography (Lin et al., this meeting). The major HPLC peak was applied to assorted potential targets to determine whether it contains physiologically activ material. Application of the main peak to the ganglion caused long lasting hyperpolarization of R3-R14, R16 and several other cells in the right lower ventral quadrant. The normal spontaneous firing of R3-R14 was inhibited by the peptide, suggesting a negative feedback system which shuts off the neurons when released peptide reaches a certain level. Most Aplysia neurons are unaffected by R3-R14 peptide. Perfusion through a beating heart increased the frequency and amplitude of contractions of the ventricle. R3-R14 peptide causes contraction of the gastroesophageal but not the abdominal and anterior aortas of Aplysia. The threshold is below the amount of material in one cell body. R3-R14 peptide also causes contraction of strips of the ventricular myocardium with a threshold of about the amount of material obtained from 0.05 cell bodies (ca. 5 ng). Strips of crop muscle are also caused to contract with a threshold of the amount of material from 0.25 cell bodies. The effects of glycine and R3-R14 peptide on the gastroesophageal artery augment each other, while the peptide overcomes the depolarizing effect of glycine on R3-R14 cell bodies. The effects of R3-R14 peptide reported here together with considerable evidence that R3-R14 utilize glycine as an intercellular messenger (Sawada et al., 1981), makes it highly probable that R3-R14 release multiple substances to act as chemical messengers.

Coggeshall, R.E., Kandel, E.R., Kupfermann, I. and Waziri, R., J. Cell Biol. 31: 363-368 (1966).
Nambu, J.R., Taussig, R., Mahon, A.C. and Scheller, R.H., Cell 35: 47-56 (1983).
Sawada, M., McAdoo, D.J., Blankenship, J.E. and Price, C.H., Brain Res. 207: 486-490 (1981).

203.10 AMINO ACID INCORPORATION INTO PEPTIDES IN APLYSIA NEURONS R3-R14. C.Y. Lin* and D.J. McAdoo. Marine Biomedical Institute, Univ. of Texas Med. Br., Galveston, TX 77550.

Aplysia neurons R3-R14 are characterized by high concentrations of free glycine. Although we have previously presented evidence that R3-R14 may utilize glycine as a neurochemical messenger, it has recently become apparent that the peptides manufactured by R3-R14 are also very glycine rich (Nambu et al., 1983). In order to explore whether the high concentrations and high rate of glycine uptake in R3-R14 are primarily to support peptide synthesis, we have compared the amounts of free and peptide-incorporated amino acids and the rates of uptake of several amino acids into R3-R14 and their incorporation into R3-R14 peptides (Table 1).

Table 1. Presence, uptake and incorporation of amino acids into R3-R14 peptides.

Amino Acid	Free AA/cell[a] nmol/cell	Inc./Free[b]	Inc./Uptake[c]
Glycine	1.6	0.09	0.0035
Alanine	0.26	0.34	<.0004
Methionine	0.008	1.5	0.13
Histidine	0.05	1.9	0.12
Arginine	0.13	0.5	0.005
Aspartate	1.0		

a. Based on an analysis of the free amino acids present in a pool of 50 R3-R14 cell bodies.
b. Based on an amino acid analysis of the major HPLC peptide peak obtained from 90 R3-R14 cell bodies.
c. Based on the amounts of radioactivity incorporated into a major and minor HPLC peptide peak from R3-R14 and the amounts in free amino acids in the cell.

According to the data in Table 1, a smaller fraction of the glycine in R3-R14 is incorporated into peptide than any of the other amino acids examined. Similarly, relatively small fraction of the glycine taken up is incorporated into R3-R14 peptides. This suggests that glycine levels are high in R3-R14 to serve some function other than to support peptide synthesis. We have presented considerable evidence (Sawada et al., 1981 and references therein) that R3-R14 may utilize glycine as an intercellular messenger.

Nambu, J.R., Taussig, R., Mahon, A.C. and Scheller, R.H. Cell 35: 47-56 (1983).
Sawada, M., McAdoo, D.J., Blankenship, J.E. and Price, C.H. Brain Res. 207: 486-490 (1981).

203.11 IMMUNOPEROXIDASE MASKING OF IMMUNOFLUORESCENCE PROVIDES AN INDEX OF THE COEXISTENCE OF SEROTONIN, SUBSTANCE P AND ENKEPHALIN IN AXONAL ENDINGS IN THE VENTRAL HORN OF THE SPINAL CORD. M.A. Ruda. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, Maryland 20205.

Numerous studies have examined coexistence in neuronal cell bodies. The demonstration of coexistence in axons has had limited technical successs mainly due to non-specific staining of the first antigen with the second chromagen. Using a combination of PAP and indirect fluorescence immunocytochemical techniques, a double label method for identifying the location and density of axons which contain coexistent neurotransmitters is described. Serotonin (5-HT), substance P (SP) and enkephalin (ENK) axons in the cat lumbar ventral horn were selected for study since brain stem neurons containing coexistent neurotransmitters contribute afferents to the spinal cord. In frozen sections, the first antigen was labeled with the PAP technique. The second antigen was labeled with immunofluorescence (IgG/FITC or IgG/Rhodamine). Both primary antisera (Immunonuclear) were produced in the same species. As a control, the same antigen was sequentially processed for both PAP and immunofluorescence or twice for immunofluorescence using different fluorochromes. Sections in which the same antigen was labeled sequentially with PAP and fluorescence contained no fluorescent axons. Sections double labeled with immunofluorescence exhibited all axons labeled by both fluorochromes. Thus, the PAP reaction product appeared to mask the second fluorescent label. In the ventral horn the number of 5-HT axons was greatest; SP noticeably fewer and ENK the fewest. Double labeled sections in which 5-HT axons were labeled with PAP contained only an occasional fluorescent SP axon, while the density of fluorescent ENK axons was similar to control, ruling out non-specific effects of PAP on immmunoreactivity. When SP axons were labeled with PAP, a small decrease in fluorescent 5-HT axons was apparent, especially around motorneurons, while the density of fluorescent ENK axons was similar to control. When ENK axons were labeled with PAP, no noticeable difference in the density of either 5-HT or SP fluorescently labeled axons was observed. These observations suggest that almost all ventral horn SP axons also contain 5-HT while most of the 5-HT axons contain neither SP nor ENK. The combination of PAP immunocytochemistry and immunofluorescence thus provides a method for identifying the location and density of axons with coexistent neurotransmitters.

203.12 PROCESSING BY OPIOMELANOTROPINERGIC NEURONS PROFOUNDLY AFFECTS BEHAVIORAL ACTIONS OF SECRETED PEPTIDES. M. D. Hirsch* and T. L. O'Donohue (SPON: W. Mink). Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205.

Opiomelanotropinergic (POMC) neurons in mammalian brain and endocrine cells in intermediate lobe of the pituitary synthesize and secrete peptides derived from a single prohormone. The phenomena of synthesis and secretion of multiple peptides by an individual cell leads one to hypothesize that the functions of these peptides may be interrelated. For example, it has recently been shown that the non-opioid C-terminus of B-endorphin 1-31 (BE) modulates the chromogenic response of melanocytes to alpha-melanocyte-stimulating hormone (α-MSH) (cf. Logan, et al., Peptides 2:121, 1981). To further test the hypothesis of a functional relationship between POMC-derived α-MSH and BE peptides, we investigated their pharmacological interactions.

Adult, male Sprague-Dawley rats (250-350 g) received intracerebroventricular (ICV) administration of peptide solutions containing varying dose-combinations of α-MSH and acetylated and des-acetyl forms of BE 1-31 (human) and fragments. Control solutions contained saline vehicle in place of respective peptides. Excessive grooming, stretch-yawn-syndrome (SYS), and catatonic behaviors were quantitated over a 55 min. period as described previously (Gispen, et al. Life Sci. 17:645, 1975).

The results indicated that both α-MSH and BE 1-31 induced dose-related excessive grooming behavior. The potency of α-MSH was increased by acetylation, while the potency of BE 1-31 was markedly decreased by acetylation. The effects of α-MSH and BE 1-31 were distinguishable by the fact that α-MSH also induced SYS but not catatonia, while BE 1-31 also induced catatonia but not SYS. In addition, BE 1-31 significantly inhibited α-MSH-induced grooming and SYS, while α-MSH significantly increased the duration of BE 1-31 catatonia. Acetylation reduced the ability of BE 1-31 to inhibit α-MSH's actions. Surprisingly, cleavage of BE 1-31 to BE 1-27 resulted in a peptide which induced SYS, as did BE 1-26 which also lacks the C-terminus. Consistently, this C-terminal fragment BE 28-31 inhibited α-MSH induced grooming and SYS in a dose-related manner, as did BE 6-31 which contains this C-terminal tetrapeptide. In addition, BE 28-31 inhibited BE 1-27 grooming and SYS, but did not effect BE 1-31 grooming. Interestingly, structural differences in the C-terminus of rat and human BE 1-31 altered catatonic potency, but did not affect grooming potency or α-MSH antagonism. These results indicate that processing of peptides by POMC neurons profoundly affects behavioral actions of the secreted peptides.

203.13 COEXISTENCE OF PUTATIVE TRANSMITTERS IN RAPHE NEURONES TRANSPLANTED TO THE RAT STRIATUM. M. Schultzberg*G.A.Foster*, F. Gage, A. Björklund*and T. Hökfelt*(SPON: H. Aldskogius). Dept. of Histology, Karolinska Institutet, P.O. Box 60 400, S-104 01 Stockholm, Sweden.

The occurrence of more than one putative neurotransmitter within the same neurone has been demonstrated in many parts of the central and peripheral nervous systems. One example is the medulla oblongata, where some Raphe neurones have been shown to contain 5-hydroxytryptamine (5-HT), substance P and thyrotropin releasing hormone (TRH). The ability of these neuronal cell types to survive and send out processes was studied in intracerebral transplants. The effect on the phenotypic expression of changing the environment of these neurones was also investigated. Suspensions of fetal rat (embryonic day 15) brain tissue were injected stereotaxically into the adult rat striatum, in which serotonergic afferents were previously removed using 5,7-dihydroxytryptamine. The rats were taken for immunohistochemical studies at least six weeks after grafting. Survival of 5-HT, substance P and TRH immunoreactive cells, and cells with various permutations of these substances were observed in the transplants. No obvious outgrowth of fibres from the grafts was seen, but networks of fibres containing 5-HT, substance P- and to a lesser extent TRH-like immunoreactivity could be seen within the transplants. Upon comparison of consecutive sections, 5-HT, substance P and TRH immunoreactive fibres had a similar localization.

In conclusion, medullary Raphe neurones containing 5-HT, substance P- and TRH-like immunoreactivity survive transplantation and are able to send out nerve processes. However, they do not seem to reinnervate the previously denervated host striatum, which may reflect the fact that it is not the normal projection area of these cells. It also appears that the phenotypic expression of the transplanted medullary Raphe neurones is independent of both their cellular milieu and the environment of their axonal/terminal processes.

203.14 SPINAL CORD 5-HT_1 BINDING SITES APPEAR COUPLED TO SUBSTANCE P BINDING SITES. F.P. Zemlan and M.M. Behbehani. Dept. of Psychiatry, Physiology and Biophysics, University of Cincinnati School of Medicine, Cincinnati, OH 45267-0559.

Substance P (SP) and Serotonin (5-HT) have been shown to coexist in the same bulbospinal neurons and have been colocalized in the same dense core synaptic vesicles in the spinal cord. These data suggest that 5-HT and SP are coreleased. The present study investigated whether SP modulates the binding of H^3-5-HT to 5-HT_1 binding sites in rat spinal cord. ^{3}H-5-HT (2nM) binding to spinal cord dorsal or ventral horn membranes was determined in the presence or absence of unlabeled 5-HT (2uM).

Addition of SP to the incubation media resulted in an increase in ^{3}H-5-HT binding in both dorsal and ventral horn. Addition of 10 nM SP produced about a 10% increase in specific ^{3}H-5-HT binding in both regions of the spinal cord (p's < 0.03). Maximal ^{3}H-5-HT binding was observed at 1 to 10 uM SP where specific binding increased about 30% (p's < 0.001). The effect of SP on ^{3}H-5-HT binding [2nM] was dose-response related:

Specific ^{3}H-5-HT Binding (fmoles/mg)

[SP]	Dorsal Horn	Ventral Horn
0nM	17.08	13.42
10nM	18.55	14.99
100nM	18.10	14.63
1000nM	19.66	17.26
10000nM	23.08	—

Mean ^{3}H-5-HT binding in spinal cord determined from 3 to 7 assays performed in triplicate. The specificity of the presently reported enhanced 5-HT binding in the presence of SP as well as the binding kinetics will be discussed. (Supported by USPHS Grant NS18326).

203.15 ACETYLCHOLINESTERASE AND SOMATOSTATIN-IMMUNOREACTIVITY COEXIST IN NEURONS IN RAT CEREBRAL CORTEX AND HIPPOCAMPUS, BUT NOT IN CH4 CHOLINERGIC NEURONS OF THE BASAL FOREBRAIN. C. H. ZHU*, J. R. DELFS, E. J. MUFSON, M. A. DICHTER, AND M-M. MESULAM. DIVISION OF NEUROSCIENCE, CHILDREN'S HOSP., AND DEPT. OF NEUROLOGY, BETH ISRAEL HOSP., BOSTON, MA 02115.

There are profound and relatively selective decreases of cholinergic markers including choline acetyltransferase (ChAT) and acetylcholinesterase activity (AChE), and of somatostatin-immunoreactivity (SOM-IR) in brains of persons with Alzheimer's disease (Bowen et al. 1976, Davies and Maloney 1976, Perry et al. 1977, Davies et al. 1980, Rossor et al. 1980). We have recently reported that AChE and SOM-IR coexist in neurons cultured from rat cerebrum (Delfs et al., Science 223: 61-63, 1984). The present study, based on methods for the concurrent demonstration of AChE and SOM-IR (Levey et al. 1983), examines the relationship between these two markers in the intact rat brain.

Intensely staining AChE-rich neurons were seen in all of the basal-forebrain cholinergic nuclei. Less intensely staining AChE-positive cell bodies were also present throughout cortex. Staining for SOM-IR (antibody gift of S. Reichlin) was seen in neurons throughout the brain and was largely consistent with reports in the literature. However, no SOM-IR was detected in the AChE-rich neurons of the Ch4 sector of the basal forebrain, known to be the predominant source of cholinergic innervation to the neocortex and amygdala (Mesulam et al. 1983).

In cortex, SOM-IR appeared to be present in many of the AChE-reactive neurons, particularly in the deeper cortical layers. On the other hand, many neurons stained only for SOM-IR. Similar findings were noted in the hippocampus.

These observations show that SOM-IR and AChE do coexist in many neurons, confirming our findings in cell culture.

Since recent evidence suggests that there may be little, if any, overlap between AChE-reactivity and ChAT-positive neurons in cortex (Levey, Rye, Wainer, Mufson, and Mesulam, Neurosci., in press), the overlap of SOM-IR and AChE cannot be interpreted to indicate the coexistence of acetylcholine and somatostatin in cortical neurons. However, the AChE-positive staining raises the possibility that a subset of somatostatinergic neurons could be cholinoceptive.

These results also demonstrate that SOM-IR is not found in those neurons responsible for the major cholinergic innervation of the cortical mantle, results which are compatible with lesioning studies (McKinney et al. 1982).

203.16 MUTUALLY EXCLUSIVE LOCALIZATION OF IMMUNOREACTIVE OXYTOCIN AND VASOPRESSIN IN THE MEDIAN EMINENCE OF THE CAT AND CO-LOCALIZATION OF IMMUNOREACTIVE VASOPRESSIN WITH MET- AND LEU-ENKEPHALIN. H. D. Coulter, R. Elde, K. Hogquist*, and F. K. Roche*. Department of Anatomy, University of Minnesota, Minneapolis, MN 55455

Blocks of median eminence from male and female prepubertal cats were quick-frozen at liquid helium temperature, dried in a vacuum, fixed with osmium tetroxide vapor, and infiltrated with epoxy resin. Serial sections, each about 0.15 µm thickness, were placed on teflon coated glass slides containing 80 wells per slide. In a typical experiment 8 numbered serial sections from each block were immunocytochemically stained with antibodies to vasopressin (#1), met-enkephalin (#2), leu-enkephalin (#3), and oxytocin (#4), followed by FITC- labeled second antibodies. Sections #5, 6, 7 and 8 were treated with the same antisera absorbed with their respective peptides. A monoclonal antibody specific to vasopressin was obtained from A. Hou-Yu and E. A. Zimmerman, and a rabbit antibody specific to oxytocin was obtained from Immunonuclear, Inc. Four L-enkephalin antibodies and five M-enkephalin antibodies, all raised in rabbits, were obtained from Immunonuclear, Immunotech, Bioproducts, Cambridge Research Biochemicals, and Merseyside Laboratories, and were used without purification. Fluorescence images were intensified with a silicon-intensified-tube television camera and photographic records were obtained directly from the screen of a video monitor. Immunoreactive (IR) oxytocin was found in 0.5-2 µm axons and IR vasopressin in 0.5-10 µm axons, always separately. In the internal lamina of the median eminence IR vasopressin was always found co-localized with both IR met- and leu-enkephalin. In the external lamina and subependymal layer IR enkephalin was sometimes found unassociated with IR vasopressin. Since axons of the internal lamina of the median eminence terminate in the posterior pituitary, the present findings suggest that the fibers IR for both vasopressin and enkephalin are the source of endogenous opiates which act upon the abundent opiate receptors in the posterior pituitary.

Supported by NSF grant BNS 81-19552 and NIH grant RO1 NS 19428.

203.17 AN IMMUNOFLUORESCENCE METHOD FOR VISUALIZING NEUROTRANSMITTER COEXISTENCE IN FIBERS AND TERMINALS by R.P. Elde and M.W. Wessendorf, Dept. Anatomy, Univ. Minnesota, Minneapolis, MN 55455

There are several light microscopic methods presently in use for demonstrating neurotransmitter coexistence; however none allow its visualization in fibers and terminal fields. This abstract describes a method for doing so.

The method used to show coexistence was identical to that used for immunohistochemical localization of one antigen, except that 2 immunologically distinct primary-secondary antibody systems labeled with 2 different fluorochromes were used simultaneously. An antiserum to serotonin (5HT, Immunonuclear) generated in goat was added to an equal measure of rabbit-generated antiserum to substance P (SP), and 98 measures PBS/0.3% Triton X-100 were added to make a final dilution of 1:100. This antisera mixture was applied to 10 micron cryostat sections of rat CNS and incubated overnight at 4° C. After rinsing in PBS, a mixture of secondary antibodies was applied to the tissue. This consisted of one measure of FITC-labeled swine-anti-rabbit IgG (Dako/Accurate) mixed with one measure of TRITC-labeled swine-anti-goat IgG (Tago) and 6 measures of PBS/Triton, to make a final dilution of 1:8. After incubating with the tissue for 1 hour at room temperature, the tissue was rinsed and coverslipped with PBS/glycerin 1:3. Slides were examined for immunostaining using 25X and 40X objectives on a Zeiss Standard fluorescence microscope equipped for blue and green reflected illumination and having a 560 nm interference barrier filter.

Using this technique, single fibers in the brainstem and spinal cord were found fluorescing both red and green after staining for 5HT and SP. While some of these were in white matter, others in gray matter appeared to be in apposition to neuronal somata, and therefore may be nerve terminals.

Controls for secondary antiserum cross-reactivity were negative. Likewise, when the barrier filter was used appropriately, neither blue excitation of TRITC-labeled tissue nor green excitation of FITC-labeled tissue produced any staining. This suggests that double labeling is not the result of wide-spectrum emmission by the fluorescent labels.

It is concluded that this technique is capable of visualizing coexistence of 5HT with SP in nerve fibers and terminals. It appears to be applicable to other systems.

These studies were supported by DA 05226 and DA 02148.

203.18 DISTRIBUTION OF SPINAL FIBERS AND TERMINALS IN WHICH SEROTONIN (5HT) AND SUBSTANCE P (SP) LIKE IMMUNOREACTIVITIES COEXIST by M.W. Wessendorf and R.P. Elde, Dept. Anatomy, Univ. Minnesota, Minneapolis, MN 55455

It is known that some serotonergic neurons in the B3 group also contain SP. However, investigation of the projections of these neurons has been hampered by the lack of a method for directly visualizing neurotransmitter coexistence in fibers. We have recently developed a method for visualizing nerve fibers in which 5HT-like immunoreactivity (5HT-LI) coexists with immunoreactivity for a second neurotransmitter. The present abstract reports the distribution in the rat spinal cord of fibers in which 5HT-LI coexists with SP-like immunoreactivity (SP-LI).

To examine the distribution of fibers containing 5HT and SP, male Sprague Dawley-derived rats (Holtzman) were killed by perfusion with Zamboni fixative, and spinal segments C6-8, T6-8, L4-6, and S1-3 were removed. Ten micron cryostat sections were cut in the horizontal, saggital, and transverse planes, and a solution of goat-anti-5HT mixed with rabbit-anti-SP was applied to the tissue. This incubation was followed by an application of a solution of secondary antibodies: FITC swine-anti-rabbit mixed with TRITC-swine-anti-goat. Using this protocol, fibers containing 5HT-LI fluoresced red, while fibers containing SP-LI fluoresced green.

Fibers containing both 5HT-LI and SP-LI were found in the intermediolateral cell column of the thoracic spinal cord, and in the ventral horn and around the central canal at all levels. In the ventral horn, the density of these fibers was lowest in the cervical spinal cord, and it was of intermediate density at the thoracic and lumbar levels. The highest density of these fibers in the entire spinal cord was found in the sacral ventral horn, where many of these fibers were in close proximity to very large cells resembling alpha motor neurons. Except in ventral nucleus proprius, few if any fibers were found in the dorsal horn in which 5HT-LI and SP-LI coexisted.

It is concluded that serotonergic fibers in which SP coexists are a common feature of the rat spinal cord. It appears that the function of these fibers could be studied most directly in the motor neurons of the sacral spinal cord.

These studies were supported by DA 05226 and DA 02148.

203.19 SYNAPSES BETWEEN ENTERIC SEROTONERGIC AND SUBSTANCE P IMMUNOREACTIVE NEURONS: LITTLE CO-LOCALIZATION OF THE TWO TRANSMITTERS IN THE MYENTERIC PLEXUS. M.D. Gershon and D. Sherman.* Dept. of Anat. and Cell Biol., Columbia Univ. P&S, New York, NY 10032.

Both serotonin (5-HT) and substance P (SP) are found in intrinsic neurons in the enteric nervous system (ENS). These two substances have been found to be co-localized in some neurons of the brain. Immunocytochemical evidence has been reported that suggests that they may also be co-localized in the cell bodies of enteric neurons. In order to investigate the possible co-existence of 5-HT and SP in enteric neurons we have studied the immunocytochemical localization of both substances in the myenteric plexus of the guinea pig and mouse small intestines. In addition, we have combined the electron microscopic (EM) radioautographic detection of serotonergic neurites with the EM immunocytochemical demonstration of SP. At the light microscopic level, primary antisera to 5-HT and SP from different species were used and the respective antigens were simultaneously localized by immunofluorescence using appropriately labeled secondary antisera. Colchicine pretreatment of animals (5 mg/kg) enhanced SP immunoreactivity of perikarya. Co-localization in the same cell body was extremely rare. The overwhelming majority of immunoreactive perikarya had either SP or 5-HT but not both; however, SP cells were sometimes surrounded by serotonergic varicosities and, more often, 5-HT cells were surrounded by SP terminals. For EM, mice were injected ip with 2 ml of 10 uM ^{3}H-5-HT and, after 2 hours, tissues were fixed with a mixture containing 4% acrolein, 0.05% glutaraldehyde, 1% formaldehyde, and 15% picric acid. SP immunoreactivity was demonstrated by pre-embedding staining in dissected myenteric plexus permeabilized by brief exposure to 95% ethanol at -20°C. Almost no terminals were found that displayed both SP immunoreactivity and radioautographic labeling by ^{3}H-5-HT; nevertheless, many terminal varicosities were seen that displayed one or the other marker in the same tissue sections. Most SP cell bodies received SP synapses and a few also received ^{3}H-5-HT terminals labeled by ^{3}H-5-HT. Perikarya that took up ^{3}H-5-HT often were contacted by SP synapses. It is concluded that co-localization of 5-HT and SP in the ENS, especially in axon terminals, is extremely rare and thus of doubtful physiological significance; however, synaptic interactions between neurons utilizing these transmitters is common and may be of considerable importance.

Supported by NIH grants NS 12969 and NS 15547.

NEUROTRANSMITTERS, MODULATORS: INTERACTIONS BETWEEN TRANSMITTERS II

204.1 **6-HYDROXYDOPAMINE INCREASES TRH CONCENTRATION IN REGIONS OF RAT BRAIN.** T.M. Engber*, S. Manaker and A. Winokur (SPON: M. Kreider). Depts. of Pharmacology, Biology and Psychiatry, Univ. of Penn. Sch. Med., Phila., PA 19104.

Thyrotropin-releasing hormone (TRH) is known to be widely distributed in the central nervous systems of many species, yet comparatively little is known about the regulation of TRH or the influence of other CNS neurotransmitters. Brain TRH concentrations are unaffected by a wide variety of pharmacologic and endocrine treatments. Amphetamine treatment has been reported to decrease striatal TRH levels without affecting those in other brain regions. Previous studies in our laboratory have shown that intracisternal administration of large doses of 6-hydroxydopamine increases TRH content in forebrain and posterior cortex of rat brain. The present study extends and refines these previous findings.

Male Sprague-Dawley rats (180-220 g) received a unilateral injection of 6-hydroxydopamine (50-400µg) or vehicle (0.9% NaCl, 0.2 mg/ml ascorbic acid) in a volume of 10µl into the lateral cerebral ventricle. Animals were sacrificed at 1, 3 or 6 days following injection and the brains were rapidly removed. The brains were then dissected on ice into 11 regions: olfactory bulb, spinal cord, septum-caudate, anterior cortex, cerebellum, brainstem, hypothalamus, posterior cortex, hippocampus, thalamus, and amygdala-piriform cortex. Brain regions were weighed, placed in 2.0 ml of ice-cold phosphate-buffered saline (PBS), homogenized, extracted with methanol and air-dried at 60°C. Samples were then redissolved in bovine serum albumin (BSA)-PBS, and TRH content was measured by radioimmunoassay. Norepinephrine and dopamine levels were measured by electrochemical detection following HPLC separation.

Intraventricular 6-hydroxydopamine (400µg) caused substantial increases in TRH concentration in 6 of 11 brain regions examined (olfactory bulb, septum-caudate, anterior cortex, brainstem, hippocampus and amygdala-piriform cortex). Increases in TRH concentration were greatest at 3 days post-injection; smaller increases were observed at 1 and 6 days post-injection. The magnitude of these increases ranged from 50% in the brainstem to over 400% in the hippocampus. It is not known whether these increases in TRH concentration reflect increased TRH synthesis, decreased TRH degradation or both.

These results suggest that catecholamines may play a role in the regulation of TRH in certain brain regions.

204.2 **5,7-DIHYDROXYTRYPTAMINE ALTERS TRH CONCENTRATIONS IN REGIONS OF RAT BRAIN.** P.B. Knight*, T.M. Engber*, S. Manaker and A. Winokur (SPON: M.A. Hofer). Depts. of Pharmacology, Biology and Psychiatry, Univ. of Penn. Sch. Med., Phila., PA 19104.

Thyroptropin releasing hormone (TRH) has been demonstrated to be widely distributed throughout the CNS, yet comparatively little is known about the regulation of brain TRH. Some recent reports suggest significant interactions between serotonin (5HT) and TRH. Thus, the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), which destroys 5HT nerve terminals, has been reported to decrease the TRH content of the spinal cord, septal nuclei, and nucleus accumbens. Additionally, TRH and 5HT have been co-localized within individual neurons in the spinal cord and the brainstem. We now report that 5,7-DHT treatment increases brainstem TRH, in addition to decreasing TRH concentrations in the spinal cord.

Male Sprague-Dawley rats (180-200g) were injected in the right lateral ventricle with either 400µg of 5,7-DHT in a vehicle of 0.15M NaCl and 1% ascorbate, or vehicle alone, and sacrificed at various time intervals. The brains were rapidly dissected on ice into 11 regions: olfactory bulb, spinal cord, septum-caudate, anterior cortex, cerebellum, brainstem, hypothalamus, posterior cortex, hippocampus, thalamus, and amygdala-pyriform cortex. Each region was then weighed, homogenized in 2ml ice-cold phosphate-buffered saline (PBS), extracted with 10 ml methanol, and air-dried at 60°C. Samples were then dissolved in bovine serum albumin-PBS, and TRH content determined by radioimmunoassay. Serotonin and norepinephrine were measured by electrochemical detection following HPLC separation.

5,7-DHT treatment resulted in a 50% decrease in spinal cord after 5,7-DHT treatment, and appeared no earlier. In addition, a 50% increase in brainstem TRH concentrations occurred at 7 and 14 days after 5,7-DHT treatment, which returned to control values by 21 days after treatment. TRH concentrations at 14 days after treatment were unchanged in all other brain regions. Studies are underway to fully evaluate the dose response and time course characteristics of these effects in all brian regions. It is unknown whether these alterations in brain TRH concentration are due to effects on synthesis, degradation, or transport of TRH. However, these results do suggest that indoleamines play a role in the regulation of brain TRH content.

204.3 SEROTONERGIC REGULATION OF DOPAMINE BETA-HYDROXYLASE IN THE ADRENAL GLAND. L. Lima* and T.L. Sourkes. Depts. of Psychiatry and Biochemistry, McGill Univ., Montreal, Canada H3A 1A1.

Dopamine-beta-hydroxylase (DBH, EC 1.14.2.1) catalyzes the conversion of dopamine to noradrenaline. DBH, tyrosine hydroxylase (TH) and phenethanolamine N-methyl transferase (PNMT) are all inducible in the adrenal gland. TH is under neural control; DBH and PNMT are under both neural and humoral regulation. Treatments such as restraint, cold exposure and administration of 6-hydroxydopamine, insulin and reserpine increase the activity of DBH. Reserpine increases the number of enzyme molecules through an increase in the rate of synthesis, effected by a neural pathway. The present study was carried out in order to understand the action of reserpine and the possible serotonergic regulation of adrenal DBH. Male Sprague-Dawley rats of about 200 g were used. DBH was determined by the radioenzymatic method of Molinoff et al. (1971). Surgery was performed under chloral hydrate anesthesia. Reserpine, in a dose of 2.5 mg/kg daily for 3 days, increases adrenal DBH activity 40-100% over control values, without significantly affecting the K_m for tyramine. The effect is significantly reduced, but not completely eliminated, by splanchnicotomy; it persists in hypophysectomized animals. The induction is blocked by cycloheximide. Neither p-chlorophenylalanine (PCPA), 300 mg/kg ip, nor 5,7-dihydroxytryptamine (DHT), 175 ug intracerebroventricularly, modifies the resting levels of adrenal DBH. However, both treatments potentiate the action of reserpine, PCPA yielding 40% and DHT 180% increases over the values obtained with reserpine alone. These drugs seem to act at different sites. Thus, the effect of DHT is blocked by denervation, a result suggesting that in the intact rat descending serotonergic fibers play a role in the regulation of adrenal DBH. On the other hand, the effect of PCPA on the inducing action of reserpine is not blocked by denervation and is probably due to the potent inhibition of tryptophan hydroxylase in the hypothalamus. Thus, serotonergic neurons appear to exert an inhibitory action over the effect of reserpine as inducer. This could be due to the potentiation by PCPA and DHT of the decrease in central serotonin caused by reserpine.

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204.4 INTERACTIONS BETWEEN DOPAMINE-SENSITIVE AND VIP-SENSITIVE ADENYLATE CYCLASE SYSTEMS IN RABBIT RETINAL HOMOGENATES. Jonathan A. Pachter*and Dominic Man-Kit Lam (SPON: S.F. Basinger) Program in Neuroscience and Cullen Eye Institute, Baylor College of Medicine, Houston, TX 77030.

In the rabbit retina, dopaminergic neurons have been localized to the amacrine cell layer, with processes extending into the inner plexiform layer to contact other amacrine cells (Dowling & Ehinger, J. Comp. Neurol. 180: 203). Similarly, vasoactive intestinal peptide (VIP) immunoreactivity is found in the amacrine cell layer, with projections into the inner plexiform layer (Tornqvist et al., Histochem. 76: 137; Chen et al., Soc. Neurosci. Abstr., 1984). Like dopamine, VIP has also been shown to stimulate production of cAMP in the rabbit retina (Schorderet et al., Eur. J. Pharmacol. 71: 131). The coexistence of these two transmitter-sensitive cyclase systems in the inner retina has led us to study possible interactions between them.

Dopaminergic and VIP-induced stimulation of adenylate cyclase activity were measured by monitoring conversion of (α-^{32}P)-ATP to (^{32}P)-cAMP in rabbit retinal homogenates. Maximal stimulation of cyclase activity by dopamine occurred with approximately 300 µM dopamine, while only 10 µM VIP was necessary for maximal VIP-induced stimulation. Dopaminergic and VIP-induced stimulation were found to be non-additive at both maximal and half maximal concentrations, indicating that dopamine and VIP may stimulate cAMP formation through a common adenylate cyclase complex.

The non-additivity of dopaminergic and VIP-induced stimulation does not seem to result simply from exhaustion of retinal adenylate cyclase, since 12 mM NaF and 1 mM forskolin could each stimulate greater cAMP formation than could the combination of dopamine and VIP. The specific D-2 agonist, LY141865, had no effect on VIP-induced cyclase stimulation, suggesting that the non-additivity does not result from a D-2 receptor-mediated inhibition. Finally, the dopaminergic antagonist, (+)-butaclamol blocks dopaminergic stimulation, but not VIP-induced stimulation, indicating that dopamine and VIP do not seem to be competing for the same receptor site. These results suggest that interactions between the dopamine-sensitive and VIP-sensitive adenylate cyclase systems in the rabbit retina probably occur at the level of the nucleotide regulatory unit or adenylate cyclase catalytic unit, rather than at the receptor level.

This work was supported by NIH grant EY02608, the Retina Research Foundation (Houston) and Research to Prevent Blindness, Inc. (N.Y.).

204.5 EFFECT OF RESERPINE AND DESMETHYLIMIPRAMINE ON CORTICOTROPIN-RELEASING FACTOR (CRF)-LIKE IMMUNOREACTIVITY OF RAT BRAIN NUCLEI. Y. Tizabi, G. Skofitsch* and D. M. Jacobowitz. Dept. of Pharmacology, Howard University College of Medicine, Washington, D.C. 20059 and Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

Immunocytochemical studies have revealed CRF-like immunoreactivity (CRFLI) in a number of brain regions that also contain norepinephrine. It was therefore of interest to study the possible influence of drugs that are known to affect catecholamine release and receptor reactivity on CRF concentration of various brain nuclei.

One group of male Sprague-Dawley rats weighing 200-250 g were injected with saline or reserpine (2 mg/kg i.p.) for 3 days. Another group of rats were injected with saline or desmethylimipramine (DMI) (20 mg/kg i.p.) for 14 days. Twenty four hours following the last injection, the animals were decapitated and their brains were quickly removed and frozen on dry ice. Coronal brain slices (300 µm) were cut in a cryostat, and 10 different nuclei (N) were dissected for CRF RIA. The areas studied were: N. interstitialis striae terminalis (dorsal and ventral), periventricular N., N. suprachiasmaticus, paraventricular N. (PVN), median eminence (ME), arcuate N., dorsomedial N., N. amygdaloideus centralis and median forebrain bundle. The CRF concentration was determined by RIA using a commercially available antibody and ^{125}I-ligand directed against rat CRF. Reserpine treatment resulted in a 41% decrease in the CRFLI of the ME, while there was approximately a 90% increase in the PVN concentration of CRFLI. On the other hand, DMI treatment resulted in an approximately 60% decrease of CRFLI in the PVN with a tendency for an increase in the CRFLI of the ME.

These preliminary data support the role of monoaminergic regulation of CRF release from the ME and further suggest a possible interaction of norepinephrine with CRF-containing cells in the PVN, resulting in an inhibitory effect of norepinephrine on the synthesis of CRF.

204.6 SEROTONINERGIC/NORADRENERGIC INTERACTION ON LUMBAR MOTONEURONES IN THE SPINAL CORD OF THE RAT. Louis E. Tremblay,* P.J. Bédard and R. Maheux.* Lab. Neurobiologie, Dept. Anatomie, Univ. Laval, Québec. G1J 1Z4

In the rat, the ventral spinal cord is richly innervated by 5-HT and NA terminals. The terminals degenerate after spinal cord transection. We have previously established (Brain Research, 169: 393-397, 1979) that after transection of the spinal cord, 5-HTP, a serotonin precursor administered intraperitoneally, increases the spontaneous level of EMG activity in the hindlimb muscles. This effect of 5-HTP progressively increases until the twentieth day after transection. The action of 5-HTP is antagonized by cyproheptadine.

Clonidine, an alpha$_2$ adrenergic agonist (0.1mg/kg, i.p.) induced a small but non significant burst in the first five minutes after administration followed by a significant depression in the baseline level of spontaneous EMG activity. 5-HTP (100 mg/kg) alone increased the EMG activity by 450 and 400% respectively for extensor and flexor muscles of the thigh, and this response remained stable for one hour and more. But, if clonidine (0.001-2.5 mg/kg, i.p.) was administered 10 minutes after 5-HTP, this alpha$_2$ adrenergic agonist depressed significantly the 5-HTP induced response of the spontaneous EMG activity by 30-70% in a dose-related fashion. On the other hand, clonidine (0.1 mg/kg) increased by 145% the threshold of evoked reflexes in chronic spinal rats. The antagonistic effect of clonidine on the action of 5-HTP but not the effect of cyproheptadine is abolished by pre-treatment with the alpha$_2$ adrenergic antagonist, Yohimbine (1.25 mg/kg, i.p.)

This interrelationship between the serotonergic and noradrenergic systems supports the possibility of balance-like interaction between 5-HT and NA on the lumbar spinal motor system. (Supported by MRC of Canada).

204.7 BEHAVIORAL AND NEUROCHEMICAL INTERACTIONS OF PHENCYCLIDINE (PCP) AND HALOPERIDOL OR AMPHETAMINE IN KITTENS. D.S. Schwartz‡ M. Allen‡ J.H. Hannigan, S. Howard-Butcher and M.S. Levine (SPON: M.E. Melnick). Mental Retardation Research Center and Dept. of Pharmacology, UCLA Los Angeles, CA 90024.

This experiment was designed to assess some of the behavioral and neurochemical interactions between PCP and drugs that alter dopaminergic (DA) neurotransmission. Kittens received pretreatment with haloperidol (HAL) or amphetamine (AMPH) before receiving injections of PCP. To date, fourteen kittens (30-35 days of age) have been tested. Five received HAL (6 mg/kg s.c.) 30 min before PCP (2 mg/kg s.c.), 5 received AMP (5 mg/kg i.p.) 45 min before PCP and 4 received saline 30 min before PCP. Behavior was assessed for changes in motor activity, posture and stereotypic head movements before and after HAL, AMPH and saline injections and for 30 min after PCP. Following PCP injections behavior was rated on a 6 point scale which quantified the intensity of the PCP effects. Periodically, throughout the session the kittens were also given a neurological assessment (muscle tone, pupil dilation, righting reflex). At the end of the behavioral testing the kittens were sacrificed, the brains quickly removed and the caudate nuclei dissected for determination of DA levels using HPLC. Kittens treated with AMPH increased activity and stereotypic head movements for the 45 min pretreatment period relative to both saline-and HAL-treated animals. HAL-treated kittens decreased activity and lost control of limbs leading to postural and motor disability within 10 min. PCP injections after saline pretreatment produced ataxia, staggering and loss of limb support. Immediately following PCP injections HAL-pretreated animals displayed higher PCP ratings than did animals in the other two groups. Within 10 min of PCP injections the elevated activity and stereotypies in the AMPH-pretreated groups were no longer observed. AMPH-pretreated and saline-pretreated animals displayed similar time courses of PCP effects but AMPH pretreated animals had lower PCP intensity ratings than animals in the other groups at the end of the 30 min post-PCP observation session. Caudate DA levels were decreased in kittens pretreated with HAL and were slightly elevated in AMPH pretreated kittens. Taken together these results demonstrate that both the behavioral and neurochemical effects of PCP can be modulated by alterations in DA neurotransmission and provide evidence that some of the effects of PCP may involve neurochemical alterations in the caudate nucleus.

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204.8 INTERACTION BETWEEN CHOLINERGIC AND ADRENERGIC RECEPTOR STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IN RAT BRAIN SLICES. R.A. Gonzales and F.T. Crews, Dept. of Pharmacology, University of Florida School of Medicine, Gainesville, FL 32610.

Recent studies have shown that phosphoinositide hydrolysis is coupled to several neurotransmitter receptor systems in the central nervous system. The muscarinic (M_1) cholinergic receptor and the alpha$_1$ adrenergic receptor have both been shown to stimulate the release of inositol phosphates from inositides. We have investigated in detail the interaction between the cholinergic and adrenergic receptor stimulation of inositide hydrolysis in Sprague-Dawley brain slices. Cortical slices (0.35 mm cubes) were prepared, washed, and incubated with [^{3}H]inositol for 90 min. The labelled slices were then washed, distributed into tubes, and exposed to various agonists and antagonists for 60 min. Incubations were terminated by addition of 1 ml of chloroform-methanol (1:2). After extraction of lipids and separation of phases, inositol phosphate was determined by Dowex-1 chromatography. Muscarinic antagonists, atropine and pirenzepine, blocked carbachol stimulated release of inositol phosphates but not that stimulated by norepinephrine. The alpha$_1$ antagonist prazosin (1 µM) blocked norepinephrine stimulation while the alpha$_2$ antagonist yohimbine (1 µM) did not. Propanolol had no effect on inositol phosphate release stimulated by carbachol or norepinephrine. The time course of inositol phosphate release in the presence of maximal carbachol reached a plateau after 30 min of stimulation. The norepinephrine stimulated response however did not plateau until 60 min. The combination exhibited characteristics of both. Dose response curves revealed that norepinephrine had an ED_{50} of 10 µM compared to ED_{50} of 100 µM for carbachol. The maximal responses were approximately equal. When a moderate concentration of carbachol was added along with norepinephrine, an additive response was seen. However, at maximal concentrations of each, the response was less than additive. The data suggest that low concentrations of muscarinic or alpha$_1$ agonists probably act via different receptor systems with little or no interaction. However, at higher concentrations, there may be a small population of cells which are activated which contain a common pool of inositides which are hydrolyzed. (Supported by N.I.A.A.A. No. AA06069).

204.9 **MODULATORY ACTION OF SEROTONIN ON GLUTAMATE-INDUCED EXCITATIONS ON CEREBELLAR PURKINJE CELLS.** Munhyang Lee, Jean C. Strahlendorf, and Howard K. Strahlendorf. Physiology and Medical and Surgical Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Many anatomical studies have revealed the existence of serotonin (5-hydroxytryptamine, 5-HT)-containing afferent fibers in the cerebellum. In the molecular layer of the cerebellar cortex, 5-HT-containing fibers morphologically resemble parallel fibers that presumably use glutamate as a neurotransmitter. Because of the close anatomical proximity of these two fiber systems, we wanted to examine the influence of serotonin on glutamate-induced responses of Purkinje cells. Pulsatile iontophoretic applications of glutamate (0-25 nA, 20 sec) at regular intervals (80 sec) produced consistent increases in the Purkinje cell discharge rate. 5-HT (5-40 nA), applied continuously with currents that induced minimum changes in the spontaneous rate, profoundly influenced glutamate-induced excitations. Specifically, serotonin completely blocked glutamate-induced excitations in 4 out of 95 cases, markedly decreased glutamate responses of 83 neurons, had no effect on 5 neurons, and potentiated glutamate excitations in 3 cases. Thus, 5-HT decreased glutamate-induced excitations in 87% of all trials and in 4% of cases blocked the glutamate excitations in the absence of equivalent effects on spontaneous firing rates. One of the interesting findings was that the direction of the effect of serotonin on the spontaneous firing rate of Purkinje cells did not appear to influence its interaction with glutamate-induced excitations. Among 77 neurons in which the effects of serotonin on spontaneous firing rates of Purkinje cells were evaluated before glutamate application, 5-HT-induced three different effects: inhibition, biphasic effect, and excitation, as shown previously from this laboratory (Brain Research Bulletin, vol. 11, 265-269, 1983). However, the inhibitory modulating effect of serotonin on glutamate responses on Purkinje cells was not related to direct effects of serotonin on Purkinje neurons. Currently, we are trying to identify the serotonin receptor subtype that may be responsible for these modulatory effects of serotonin on glutamate-induced excitations. On occasions, methysergide, a 5-HT antagonist, blocked the modulatory inhibitory effects of 5-HT on glutamate-induced excitations of Purkinje cells. (Supported by Tarbox Parkinson's Disease Institute, TTU and NIH R01 NS 19296)

204.10 ACTION OF DOPAMINERGIC AGONISTS ON THE IN VIVO NEO SYNTHESIZED ^{3}H-GABA RELEASE IN THE RAT STRIATUM. M.J. Besson*, J.A. Girault*, U. Spampinato*, J. Glowinski* and H.E. Savaki* (SPON: G. Barbin) Chaire de Neuropharmacologie, INSERM U.114, Collège de France, 75231 Paris cedex 05, France.

The interaction between dopaminergic and GABAergic neurons was investigated at the rat striatal level by measuring the in vivo release of neosynthesized ^{3}H-GABA, a biochemical index of GABAergic neurons' activity. A push-pull cannula was implanted in the striatum in rats anesthetized under a mixture of halothane nitrous oxide O_2. The tissue was superfused with artificial CSF enriched with ^{3}H-glutamine, to label continuously GABAergic neurons. One hour after the onset of superfusion serial 10min superfusate fractions were collected for a 180min period of time. The resting release of ^{3}H-GABA was decreased when the superfusing CSF was replaced by a Ca^{2+}-free and Mg^{2+}- enriched medium. Addition of K^+ ions (20mM) evoked an increased release of ^{3}H-GABA, which was partly Ca^{2+} dependent.

The investigation of dopaminergic and GABAergic neurons' interaction was performed by adding various dopaminergic agonists into the superfusing CSF during 30min and estimating the induced changes of ^{3}H-GABA release. ^{3}H-GABA release was unchanged by ADTN at several concentrations (1, 10 and 100µM). In the presence of the D_2 agonist, Ru 24926 (50 and 100µM), the release of ^{3}H-GABA was reduced in a dose related manner whereas the drug was without effect at 1µM concentration. The decreased release of ^{3}H-GABA induced by 100µM Ru 24926 was prevented by S-sulpiride pretreatment (10µM, applied 30min prior to Ru 24926 addition), S-sulpiride by itself having no effect. Acetylcholine in the presence of eserine (both at 50µM) had no effect on resting striatal ^{3}H-GABA release, although it potentiated the Ru 24926 (100µM) induced diminution, suggesting that acetylcholine can modify the D_2 dopaminergic mediated effect on GABAergic striatal neurons. The striatal effect of amphetamine (10µM) was examined following the local application of S-sulpiride (10µM) in an attempt to analyze the implication of D_1 dopaminergic receptors in the regulation of GABA release.

204.11 INTERACTION BETWEEN SOMATOSTATIN AND CHOLINERGIC MUSCARINIC RECEPTORS IN THE HIPPOCAMPUS. R. Miyoshi*, S. Kito, K. Mizuno* and K. Nitta*. Third Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima, Japan, 734.

Recently cholinergic theory of senile dementia has been advocated, whereas complex neuronal networks within the hippocampus is considered to be related with memory mechanism. In this paper, we studied on interaction between somatostatin and cholinergic muscarinic receptors in the hippocampus from viewpoints of binding experiments.

Muscarinic acetylcholine binding experiments were performed with use of ^{3}H-oxotremorine-M acetate (^{3}H-oxo-M), an agonist, as radioactive ligand and 10^{-4}M acetylcholine as non-radioactive ligand. The P_2 fraction of the rat hippocampus was prepared. Aliquots of tissue preparation were incubated in Krebs Henseleit (pH 7.4) at 30°C with and without 1 μM ^{8}[D-try] somatostatin. To prevent degradation of somatostatin, reagents such as pepstatin, bacitracin and bovine serum albumin were added. In association experiments, the muscarinic agonist binding at the concentration of 1 nM ^{3}H-oxo-M reached equilibrium in 8 min. At the equilibrium, presence of 1 μM somatostatin leveled down the specific ^{3}H-oxo-M binding to 65%, when compared to that without somatostatin. The incubation time of saturation analysis was set at 8 min. As the results, Kd value of ^{3}H-oxo-M binding to acetylcholine muscarinic receptors with somatostatin was 4.5 nM, while Kd value without somatostatin was 3.0 nM.

These days, interaction between classic neurotransmitters and neuropeptides has been a focus of increasing interestes in field of neuroscience. These results indicated that muscarinic receptors and somatostatin interacted in the hippocampal membrane and somatostatin was playing a role in regulating acetylcholine receptor functions.

Furthermore, we studied using ^{3}H-QNB on whether such interaction existed between antagonistic muscarinic receptor binding and somatostatin or not.

204.12 MODULATION OF ACETYLCHOLINE RELEASE FROM THE PERFUSED CAT SUPERIOR CERVICAL GANGLION BY α-ADRENOCEPTOR AGONISTS. D.M. Araujo* and B. Collier* (SPON: P. E. Braun). Dept. of Pharmacol. and Ther., McGill University, McIntyre Medical Bldg., Montreal, Quebec, Canada H3G 1Y6.

Since the initial finding by Marrazzi (J. Pharmacol. Exp. Ther., 65: 395, 1939) that epinephrine depressed the postsynaptic potential of the cat superior cervical ganglion, a modulatory role on ganglionic transmission for the catecholamines has been proposed. The catecholamines have been postulated to exert inhibition of transmitter release by acting on presynaptic α-adrenoceptors. The present study was undertaken to determine firstly, whether exogenous catecholamines can affect acetylcholine (ACh) release from the cat superior cervical ganglion and secondly, whether endogenous catecholamines play a modulatory role.

Perfusion of the cat superior cervical ganglion, in situ, with norepinephrine depressed evoked ACh release, at a stimulation frequency of 20Hz, with an IC_{50} of 9.7nM. Clonidine, a selective α_2-adrenoceptor agonist was less potent than norepinephrine in decreasing ACh release (IC_{50}=11.6μM). The norepinephrine-mediated inhibition of evoked ACh release was reversed by the α_2-adrenoceptor antagonist yohimbine (10nM), but was unaffected by the α_1-adrenoceptor antagonist prazosin (10^{-9}M-10^{-6}M).

To test the possible significance of endogenous catecholamine release, the superior cervical ganglion was perfused with yohimbine alone. Evoked ACh release was facilitated by yohimbine (10nM) by 24-53% (n=8). Together these results suggest a possible role for endogenous catecholamines in the regulation of ACh release from the cat superior cervical ganglion and furthermore that this modulation is mediated through an α_2-adrenoceptor.

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204.13 THE METHAMPHETAMINE-INDUCED INCREASE IN NIGRAL SUBSTANCE P-LIKE IMMUNOREACTIVITY IS MEDIATED BY THE D_2 RECEPTOR. P.K. Sonsalla*, J.W. Gibb and G.R. Hanson. Dept. Biochem. Pharmacol. & Tox., Univ. of Utah, Salt Lake City, UT 84112

It is known that the activities of the nigral-striatal dopamine (DA) pathway and the striatal-nigral substance P (SP) feedback loop are closely linked. Due to the important role of this neuronal circuitry in basal ganglia function, we have been attempting to elucidate the nature of this interaction. Recently we reported that the subacute administration of the indirect DA agonist, methamphetamine (METH), to rats increases the concentration of substance P-like immunoreactivity (SPLI) in the substantia nigra. This METH effect was blocked by the coadministration of the dopamine receptor antagonist, haloperidol (Ritter et al., J. Pharmacol. Exp. Ther., in press). These findings suggest that the METH-induced changes in nigral SPLI are mediated by the increased DA activity produced by METH. Consistent with these results, we have reported that subacute L-DOPA also increased nigral SPLI concentrations. To characterize further the nature of the dopaminergic influence on substance P activity, various specific DA agonists and/or antagonists were administered every 6 h for 4 or 5 doses. The results of these studies demonstrated that activation of the D_1 receptor with the compound, SK&F 38393 (15 mg/kg), significantly decreased nigral SPLI concentration whereas treatment with the D_2 agonist, RU 24926 (5 and 15 mg/kg), resulted in a significant increase in SPLI concentration within the substantia nigra. Blockade of D_2 receptors with sulpiride (80 mg/kg) caused a significant reduction in nigral SPLI concentrations and in combination with METH completely blocked the METH-induced changes in nigral SPLI levels. These findings demonstrate differential actions by D_1 and D_2 receptors on the striatal-nigral SP system. In addition, the data suggest that the increase in nigral SPLI concentration observed following subacute METH administration is at least partially attributable to activation of the D_2 receptor.

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204.14 OPPOSITE EFFECTS OF METHAMPHETAMINE ON SUBSTANCE P SYSTEMS IN RAT BASAL GANGLIA AND MESOLIMBIC SYSTEMS. J.K. Ritter*, C.J. Schmidt*, J.W. Gibb, and G.R. Hanson (SPON: M. Peat). Dept. Biochem. Pharmacol. & Toxicol., College of Pharmacy, Univ. of Utah, Salt Lake City, UT. 84112.

Like the dopaminergic (DA) projection of the nigral-striatal system, the DA neurons of the mesolimbic pathway are believed to respond to a substance P (SP) input which functions as part of an excitatory feedback circuit. This SP pathway projects from the medial habenula to the A10 group of DA cell bodies in the ventral tegmental area (VTA). We previously reported that the release of transmitter from the nigral-striatal DA pathway of the basal ganglia induced by subacute methamphetamine (METH) treatment results in substantial increases in the concentrations of striatal and nigral substance P-like immunoreactivity (SPLI) (Ritter et al., J. Pharmacol. Exp. Ther., in press). These observations suggest that the striatal-nigral SP pathway is partially regulated by DA activity. The present study was conducted to determine if the SP pathway associated with the mesolimbic system responds to DA activity in a similar manner.

Rats were given five sequential injections of METH (15 mg/kg, s.c.) at six-hour intervals. Eighteen hours after the final dose, SPLI concentration was reduced in both the medial habenula (48%) and the VTA (45%) with respect to saline-treated controls. These findings were opposite to the elevations in SPLI concentrations found in the striatum and substantia nigra following an identical subacute METH paradigm. However, like the METH-induced changes in the SP system of the basal ganglia, administration of the DA receptor antagonist, haloperidol (2 mg/kg), with METH completely blocked the METH-induced changes in SPLI levels in the VTA. This latter finding suggests that the change in SPLI concentration associated with the VTA results from the enhanced DA activity induced by METH.

Additional studies are needed to elucidate the significance of the METH-induced reductions in the medial habenula and VTA. One possible explanation is that these changes in SPLI concentration reflect increased release and subsequent metabolism of this neuropeptide within these structures. Interestingly, these data demonstrate that METH exerts opposite effects on the SP systems associated with the basal ganglia and mesolimbic systems.

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204.15 EFFECTS OF ELECTROCONVULSIVE SHOCK (ECS) ON THE VENTRICULAR RELEASE OF PROSTAGLANDIN (PG) IN RAT. T. Furui*, T.L. Yaksh* and S. Divinetz Romero* (SPON: Donald W. Klass) Dept. of Neurosurg. Res., Mayo Foundation, Rochester, MN 55905.

The release of arachidonic acid products following convulsions produced by electric or chemical stimuli has been shown to occur ex vivo. We have shown in vivo release of PGE_2 to respond to depolarizing stimuli in cats (Divinetz Romero et al., Brain Res., in press, 1984). Little is known of the pattern of PG release by brain tissue in vivo, and how it is affected by electrically induced seizures: this study addressed the point in a ventriculocisternal perfusion preparation in rats. Sprague-Dawley rats were anesthetized with chloralose/urethane (130 mg/kg; 1 g/kg) i.p., artificially ventilated, and prepared with a stereotaxically introduced cerebroventricular inflow cannula and a cisternal outflow cannula. Artificial cerebrospinal fluid was perfused at a rate of 100 µl/min using a two channel peristaltic pump. The outflow was collected on ice for 30-min periods and the samples frozen until organic extraction and radioimmunoassay (RIA). After a 30-min stabilization period, 2 control samples were obtained. The animals were given ECS at an intensity which would produce tonic clonic seizures in the unanesthetized animal. Poststimulation samples were collected for 1-2 hrs. The 3 main PG's and metabolites of PGI_2 and TxA_2 were measured. Prestimulation control periods were compared to the maximal elevation obtained during or immediately after the stimulation period. Statistical analysis consisted of Student's paired t-test. Experimental results are shown in the table (*$p<0.05$; pg/min):

	PGD_2	TxB_2	PGE_2	6ketoPGF$_{1\alpha}$	PGF$_{2\alpha}$
Control	17±2	13±5	12±4	9±3	8±3
Stimulated	33±9	61±24*	44±12*	31±7*	17±5*
% Change	201±65	608±92	525±184	493±128	141±13

The profile in the control period is D_2:TX:E_2:I_2>$F_{2\alpha}$. During stimulation, the profile becomes TX:E_2:D_2:I_2>$F_{2\alpha}$. Contrary to in vitro studies, the largest increases correspond to TX, E_2 and I_2, with lesser changes in PGD_2 and $PGF_{2\alpha}$. The changes in PGD_2, considered the main PG in rodents, are difficult to evaluate due to the low sensitivity of the available RIA. The results indicate that ES produces a general increase in PG efflux in vivo. However, the stimulation resulted in a preponderant increase in the relative levels of TX and PGE_2. PGE_2 has known sedative and anticonvulsant properties; scant information on physiologic effects of TX outside the vasculature makes it difficult to speculate on the overall effect of the profile changes. (Mayo Foundation & NS06663B)

BASAL GANGLIA: CELLULAR STUDIES

205.1 MORPHOLOGY OF NEURONS CONTAINING VIP-LIKE IMMUNOREACTIVITY IN THE RAT STRIATUM. E. Theriault, P.E. Marshall, and D.M.D. Landis. Neurology Service, Massachusetts General Hospital, Boston, MA 02114.

We have used light and electron microscopic immunocytochemical techniques to study the morphology of neurons in adult rat caudatoputamen containing vasoactive intestinal polypeptide-like immunoreactivity (VIP-Ir). Rats were perfused with paraformaldehyde-lysine-periodate fixative, the brain sectioned at 40um intervals on a vibratome in coronal or sagittal planes, incubated with commercially obtained anti-VIP antisera, and reacted with ABC technique. Methods controls included pre-absorption with VIP.

VIP-Ir cells were sparsely and uniformly distributed throughout the striatum. Cell bodies were 12-17um in diameter and gave rise to 3-5 primary dendrites, which branched close to the soma and could be traced up to 200um. More distal portions of the dendritic arborizations often appeared irregular and varicose. No dendritic spines were apparent at the light microscopic level. Dendrites frequently traversed bundles of myelinated axons, a pattern not exhibited by cells containing somatostatin- or Substance P-like immunoreactivity. In several instances, very fine, varicose processes arborized extensively within 40um of the cell body; these may represent axons. In more ventral and anterior striatum there were larger, axon-like arborizations far from immunoreactive cell bodies which may correspond to afferent axons from the stria terminalis.

In thin-sectioned preparations, dendrites were virtually spine free. Synapses with symmetric or asymmetric junctional specializations were present on the dendritic surface. Several VIP-Ir varicosities terminate on the cell body, forming synaptic junctions with symmetric specializations; these synapses may derive from recurrent axonal collaterals. The nucleus is deeply invaginated. VIP-Ir neurons thus resemble other aspiny striatal cells considered likely to be local circuit neurons.

205.2 CELLULAR LOCALIZATION OF THE D_1 ADENYLATE CYCLASE-LINKED DOPAMINE RECEPTOR IN THE CAUDATE NUCLEUS. M.A. Ariano & S.L. Kenny* Anatomy & Neurobiology, Univ. Vermont College of Medicine, Burlington, VT 05405.

Multiple dopamine receptors have been described pharmacologically within the caudate nucleus using a variety of experimental approaches. One dopamine receptor subtype is linked through adenylate cyclase (EC 4.6.1.1) and the production of cyclic AMP for mediation of its postsynaptic events. It has been designated D_1 (Nature 277: 93, 1979). The present study describes the D_1 receptor-containing cell population of the rat caudate-putamen complex in intact tissue slices.

Cellular localization of the D_1 receptor has been accomplished through *in vitro* binding of a radiolabeled semi-rigid analog of dopamine, ^{3}H-ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene), and subsequent autoradiographic analysis (Brain Res. 179: 255, 1979). Co-localization of cyclic AMP, using immunohistochemistry, was also used to ascertain the striatal cell population containing the D_1 adenylate cyclase-linked receptor subtype. Preliminary data demonstrates cyclic AMP immunoreactivity to be as we have previously described, using a well-characterized antisera at 1:200 dilution in phosphate buffer on 8 µm frozen sections (Neuroscience 9: 23, 1983). Visualization of immunoreactive cyclic AMP sites used the PAP method of Sternberger (1979) with 3,3'-diaminobenzidene as substrate. ^{3}H-ADTN was incubated at 1:1000 dilution for 1 hour at room temperature, followed by exposure of the sections to Kodak NTB-3 emulsion for 3 to 5 days at 4°C. The autoradiographic positive cells were 2.5 times more densely labeled than the surrounding neuropil, and 5 times more reactive than the grain density of the background emulsion under these experimental conditions.

Initial results show that approximately 40% of cyclic AMP positive elements within the striatum also demonstrate silver grains corresponding to the binding of tritiated ADTN to the D_1 receptor subtype. The majority of D_1-containing, cyclic AMP-immunoreactive cells are cytoarchitecturally similar to medium-sized perikarya of the caudate-putamen. A small amount of these ^{3}H-ADTN-cyclic AMP-reactive cells are less than 10 µm in diameter and resemble glial elements. This data suggests that the primary D_1-containing, cyclic AMP-positive components in the striatum are medium-sized neurons.

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205.3 LIGHT AND ELECTRON MICROSCOPIC LOCALIZATION OF SEROTONIN IMMUNOREACTIVITY IN NUCLEUS ACCUMBENS OF MONKEYS. Gay R. Holstein, Tauba Pasik and Pedro Pasik. Depts. Neurol. and Anat., Mount Sinai Sch. Med., CUNY, N.Y., N.Y. 10029.

Serotonin immunoreactivity has been demonstrated in nucleus accumbens of the rat (Steinbusch, 1981). To identify these fibers and their terminals at the ultrastructural level in the primate, adolescent monkeys (M. fascicularis) were perfused under deep barbiturate narcosis with 4% pure formaldehyde and 0.25% glutaraldehyde in 0.12M phosphate buffer. Some animals received an intracerebral 3 µl injection of 2.5% colchicine 16 hr before perfusion. Others were given Pargyline (75 mg/kg) and L-tryptophan (100 mg/kg) i.p., three and one hour, respectively, prior to sacrifice. Vibratome 40 µm sections were incubated in a 1:500 or 1:1000 dilution of rabbit antiserum raised against a serotonin-BSA conjugate and further processed with the PAP technique. No reaction was visible in control sections incubated with the same antiserum preabsorbed with the antigen, or coprecipitated with antigen and rabbit anti-BSA.

With light microscopy, fine processes forming a dense filigree were seen throughout the nucleus. Fiber diameters ranged from 0.3-0.8 µm. Fusiform dilations (1.2-2.5 µm long) were apparent along the thicker axons, spaced at 0.8 to 1.5 µm intervals. More spherical swellings, typically 0.75 µm in diameter, were apparent along the labeled processes, although more frequently on the finer axons. These varicosities were spaced 1.5 to 2.5 µm apart. The longer fibers tended to course dorsoventrally through the nucleus, and could be followed up to 130 µm through the neuropil. No labeled somata were visible in nucleus accumbens at either serum dilution.

Using serial section electron microscopy, numerous fibers of similar sinuous morphology were visible. Reaction was apparent in small caliber (0.3-1.4 µm diameter) myelinated as well as unmyelinated axons. Two types of reaction product were obtained: dark globular label and granular particles, the latter being typical of the large immunostained profiles. Dense core vesicles were occasionally observed, particularly in elements with globular reaction product. The diameter of labeled terminals varied from 0.6 to 1.1 µm. Some of these profiles formed asymmetric synapses with small dendritic shafts and spines.

These results provide positive identification of serotoninergic terminals in nucleus accumbens, and morphologic support for their possible excitatory action. However, the low frequency of observing synapses suggests that some portion of these afferents do not form structurally defined synaptic junctions.

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205.4 PUTATIVE PEPTIDE NEUROTRANSMITTERS LOCALIZED IN THE RAT NEOSTRIATUM. Ronald H. Bradley. Dept. of Anatomy, College of Osteopathic Medicine, Michigan State University, East Lansing, MI 48824-1316.

This laboratory has previously demonstrated at the light and E.M. level the existence of substance P, GABA and the enkephalins (met and leu) within retrogradely labelled (WGA) neostriatal projection neurons. We have utilized vibratome cut (60 µm thick) and glycol methacrylate sections (4 µm thick) to analyze the light microscope morphology of four putative peptide neurotransmitters within the colchicine-treated rat neostriatum. The four peptide neurotransmitters investigated are bombesin, cholecystokinin (CCK), somatostatin and vasoactive intestinal polypeptide (VIP) by the modified avidin:biotin technique (Bradley et al., 1984).

Somatic concentrations of putative peptide ndurotransmitters were selectively increased by bilateral lateral ventricular injections of colchicine (25 µg/µl each side) and then allowing a 48 hour survival period. The rats were then perfused with our microtubule stabilizing buffer (MSB) containing 1% glutaraldehyde - 1% paraformaldehyde, 0.1% lidocaine and 10,000 units of heparin at 37°C. The brains were immediately removed from the cranial vault and vibratome cut (60 µm) in MSB. These sections were masked with normal goat serum and then incubated with commercially available antisera (purified: albumin and complement free-IgG fraction) to bombesin, cholecystokinin, somatostatin vasoactive intestinal polypeptide, normal rabbit sera (control) or met-enkephalin (control for staining specificity) for 12 hours at 4°C with constant agitation. The sections were then extensively washed in Tris buffered saline (TBS), incubated with biotinylated IgG, rinsed with TBS, incubated with reagent peroxidase, rinsed with TBS and reacted with 0.25 mg/ml DAB with 0.001% H_2O_2. Several sections were run up for electron microscopy for further analysis.

Results indicate that bombesin, CCK, somatostatin and VIP clearly label a different population of neostriatal neurons than leu- or met-enkephalin, GABA or substance P neurons. The light microscopic data indicates a larger class of neuron (15-30 µm) as well as a medium-size neuron (10-15 µm) with distinct topographical arrangement for each peptide neurotransmitter candidate. Analyses are continuing to determine whether these are projection neurons and what are the ultrastructural characteristics of these immunopeptide labelled neostriatal neurons. (Supported by N.I.H. Biomedical Research Support Grant to M.S.U.-C.O.M.)

205.5 GLUTAMIC ACID DECARBOXYLASE, LEUCINE-ENKEPHALIN, AND SUBSTANCE-P IMMUNOREACTIVE NEURONS IN THE NEOSTRIATUM OF THE RAT AND CAT. S. Afsharpour, G.R. Penny and S.T. Kitai. Division of Neuroscience, Department of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, TN 38163

We have used immunocytochemical procedures to label cell bodies in the neostriatum for glutamic acid decarboxylase (GAD), leucine-enkephalin (Leu-enk) and substance-P (SP) immunoreactivity, with no need for pretreatment with colchicine. These experiments allow us to ask questions concerning the number, size and distribution of neurons within the neostriatum that are immunoreactive for each of these substances.

Our sample counts so far suggest that about 50% of neostriatal neurons in the rat are immunoreactive for GAD and about the same proportion are immunoreactive for Leu-enk. In this species both GAD neurons and Leu-enk neurons range from 60 to 130 um^2 in cell body cross-sectional area. Two color double labeling studies reveal that most Leu-enk neurons in the rat also display GAD immunoreactivity. However, because Leu-enk and GAD neurons together make up 60% or more of the neurons in our samples, it follows that some neurons must contain only GAD or Leu-enk, but not both.

In the cat, about 50% of neurons in the caudate are immunoreactive for GAD, and the same proportion is immunoreactive for Leu-enk. Both GAD neurons and Leu-enk neurons range from 100 to 200 um^2 in cell body area. Most Leu-enk neurons can also be double labeled for GAD immunoreactivity.

In contrast to GAD or Leu-enk immunoreactive neurons, which are fairly evenly distributed across the neostriatum, SP immunoreactive neurons are gathered into clusters that range from 250-800 um in diameter. In the cat, the SP neurons make up about 60% of the neurons within a cluster. These clusters, which are also densely filled by SP immunoreactive terminals, correspond to the enkephalin-rich patches of the neostriatum.

These results reveal a marked species similarity in the organization of GABAergic and peptidergic neurons of the neostriatum in rat and cat. Secondly, they provide another example of the coexistence of GABA with a neuroactive peptide (in other systems, motilin and somatostatin have been shown to coexist with GABA).

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205.6 VENTRAL PALLIDAL NEURONS INTRACELLULARLY LABELED WITH HRP: LIGHT MICROSCOPIC ANALYSIS. G.-X. Teng*, H. T. Chang and S. T. Kitai. (SPON: T. E. Bertorini) Dept. of Anat., Div. of Neurosci., Univ. of Tenn., Ctr. for Health Sci., Memphis, TN 38163

The ventral pallidum (VP) consisting of loosely arranged large neurons intermingled with many large myelinated fibers is located ventral to the anterior commissure and extends rostrally as the deepest layer of the olfactory tubercle (OT). Although VP is characterized by its similarities in cytoarchitectural, hodological, histochemical and immunocytochemical properties to those of the globus pallidus, little is known with regard to the detailed axonal and dendritic morphology of VP neurons. We examined several intracellularly labeled neurons located in various parts of VP to see whether the morphology or the distribution of axons and dendrites are restricted by the peculiar geometry of VP.

All labeled VP neurons had smooth somatic surface. The dendrites were mostly smooth, some had a slightly varicose appearance and occasionally had few dendritic spines or appendages. Neurons located in caudal VP had the largest dorso-ventral dendritic extent (up to 1.5mm), whereas neurons located rostrally in the fibrocellular layer of OT had the smallest dorso-ventral dendritic extent (up to .7 mm). The dendrites of all VT neurons tended to remain within the borders of VP. Few, if any, dendrites penetrated into the substance of the nucleus accumbens (NAC) dorsally, or into the intermediate layer of OT ventrally. The local axon collaterals also tended to terminate within the borders of VP. Some axons of rostrally located VP neurons, however, would course into NAC and gave rise to collaterals which coursed back into VP to terminate as beaded fine fibers. One rostral VP neuron had an axon collateral which traversed the entire deep fibrocellular layer of OT to terminate in the magnocellular zone of an island of Calleja. The main axons of all VP neurons coursed caudally. Some had rostral projecting branch coursing in the direction of the frontal cortex.

Our data indicated that the dendrites of VP neurons were bounded by the borders of the nucleus. The local axon collaterals also tended to terminate intrinsically within VP, even when their branch points were outside of VP. (Supported by NIH Grants NS20702 to S.T.K., and NRSA F-32 NS06951 to H.T.C.).

205.7 NUCLEUS ACCUMBENS NEURONS IN VITRO: RESPONSES TO LOCAL STIMULATIONS IN BRAIN SLICES. H.T. Chang and S.T. Kitai. Dept. of Anat., Div. of Neurosci., Univ. of Tenn., Ctr. for Health Sci., Memphis, TN 38163.

Passive electrical membrane properties and responses to local stimulations were studied in nucleus accumbens (NAC) neurons of the rat in IN VITRO brain slice preparations. Either frontal or parasagittal sections (0.3-0.4 mm thickness) containing NAC and its surrounding structures were used in this study. The data are from NAC neurons having stable resting membrane potentials greater than 50 mV and capable of generating action potentials greater than 60 mV. All neurons showed rectification in membrane resistance to hyperpolarizing currents greater than 0 5 nA. The input resistance and the membrane time constant (τ_D) measured from transients produced by small hyperpolarizing currents ranged from 15-40 MΩ and 5-12 msec respectively. Injections of depolarizing currents generated repetitive action potentials with a maximal frequency up to 100 Hz before inactivation. Local stimulations via a twist-pair wire electrodes evoked depolarizing post-synaptic potentials (DPSP). Increasing stimulus intensities usually triggered a single action potential on the rising phase of the DPSP. The DPSP peak amplitude could reach 40 mV. Total rise time from onset to the peak was usually less than 10 msec. The repolarization rate was slow and, upon high stimulus intensities, the falling phase of the DPSP could give rise to spikes of lower amplitudes and longer durations than the first action potential. Injections of hyperpolarizing currents increased the amplitude of the DPSP, whereas injections of depolarizing currents decreased the amplitude of the DPSP and eventually reversed a later portion of the DPSP into a hyperpolarizing potential. Superfusion of bicuculline (0.1 mM) abolished this polarity reversal. Intracellular injections of chloride ions increased the amplitude of the DPSP and decreased the rate of repolarization. These data indicated that the DPSP evoked by local stimulation in NAC consisted of a combination of excitatory and inhibitory PSP, and that the IPSP was GABAergic, probably mediated by chloride ions. All neurons (n=10) which were labeled intracellularly with HRP and had similar responses as described above were identified as medium spiny neurons. (Supported by NIH Grants NS20702 to S.T.K. and NRSA F-32 NS06951 to H.T.C.)

205.8 INTRACELLULAR RECORDING AND LABELING OF GLOBUS PALLIDUS NEURONS IN THE RAT. S. T. Kitai and H. Kita. Div. of Neuro sci., Dept. of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163.

Intracellular recordings were obtained from globus pallidus (GP) neurons following stimulation of the cerebral cortex (Cx), striatum (Str), entopeduncular nucleus (EP), subthalamic nucleus (STH) and substantia nigra (SN) in rats anesthetized with a combination of urethane (1.2g/kg) and ketamine (30-100 mg/kg). Stimulating electrodes were bipolar stainless steel insect pins insulated with epoxy to within 0.4mm of their tip with the tip separation of approximately 0.8mm. The stimulation current did not exceed 1 mA. The recording glass microelectrodes were filled with either 3M KCl or 0.5M K-methylsulfate and 3-4% HRP in 0.05M tris buffer (pH 7.6) with tip DC resistances of 30 to 120 Mohm. Some microelectrodes filled with HRP were used to stain the recorded cells in order to examine their morphological features. After recording, animals were perfused with 2% formaldehyde and 2% glutaraldehyde in phosphate buffer (PH 7.6). The brains were removed and vibratome sectioned and reacted for peroxidase histochemistry. Most of the neurons studied were antidromically activated following stimulation of either EP or STH, and some following Cx, Str, or SN stimulation. Antidromic spikes were all or none responses without underlying depolarization and they exhibited an ability to follow high frequency stimulation and IS-SD brake during hyperpolarization. All neurons analyzed had resting membrane potentials of over 40 mV and they were capable of generating spikes with amplitudes over 50 mV. Striatal stimulation induced short lasting (10-20 msec duration) EPSPs followed by IPSPs (about 50 msec). During hyperpolarization of neurons, it was observed that the onset of IPSPs almost coincided with that of the EPSPs. Postsynaptic responses from Cx, EP, STH and SN consisted of initial EPSPs (20-50 msec duration) followed by IPSPs (approximately 50 msec in duration). Microscopic analysis of the intracellularly labeled neurons revealed that the recorded GP neurons were large or medium sized with large dendritic fields. The somata and the primary dendrites were spine free. The intrinsic axon collaterals were thin and arborized partially within the dendritic field of the parent cell. In some neurons axon collaterals were traced into Str. (Supported by NIH Grant NS20702).

205.9 ENTOPEDUNCULAR NUCLEAR INPUTS TO THE LATERAL HABENULAR NUCLEUS IN THE RAT: AN INTRACELLULAR RECORDING AND LABELING STUDY. K. Yamabe, H. Kita and S. T. Kitai. (SPON: R. Caldwell). Div. of Neurosci., Dept. of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163

Anatomical studies indicate that the entopeduncular nucleus (EP) projects to the lateral habenular nucleus (LHb). In order to analyze the functional nature of this input, intracellular recordings were obtained from LHb neurons in rats anesthetized with urethane (1.2g/kg) and ketamine (30-100mg/kg). Bipolar stimulating electrodes were placed in EP, striatum (Str) and fasciculus retroflexus (Fr). Recording glass microelectrodes were filled with 0.5M K-methylsulfate and 3-4% HRP in 0.05 M tris buffer (pH 7.6) with tip DC resistances of 40-70Mohm. In order to place the recording electrode visually over LHb, the overlying parietal cortex and the dorsal hippocampus were aspirated. At the termination of experiments, animals were perfused with 2% formaldehyde and 2% glutaraldehyde. Brains were sectioned at 50 um either in sagittal or frontal plane and reacted for peroxidase histochemistry. When LHb neurons were penetrated, the microelectrode often recorded small, fluctuating spontaneous depolarizing potentials from which spike potentials were occasionally triggered. The amplitude of the depolarizing potentials was increased by injections of hyperpolarizing current which suggests that they were EPSPs. EP stimulation induced small amplitude (i.e., approximately 5 mV) depolarizations with multiple peaks having a duration of 20-50msec. These depolarizing responses were observed in about 2/3 of the neurons recorded. Responses to EP stimulation were classified into two types: One type was characterized by having a short (2-3msec) and constant latency in spite of changes in stimulus intensity. The amplitude of these responses was increased with injections of hyperpolarizing current. These results would indicate that the responses were monosynaptic EPSPs. The other type had variable latencies (3-10msec) regardless of stimulus intensity. Fr stimulation produced antidromic responses in most of the LHb neurons with latencies less than 2 msec. Thus, one of the major functions of EP projections to LHb nucleus is to directly excite neurons whose somatodendritic morphologies are currently being analyzed. (Supported by NIH Grant NS20702).

205.10 LIGHT MICROSCOPIC ANALYSIS OF RAT THALAMIC PARAFASCICULAR NUCLEUS NEURONS INTRACELLULARLY STAINED WITH HRP. H. Nakanishi, H. Kita and S. T. Kitai. (SPON: Peter K.Law) Div. of Neurosci., Dept. of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163

Neurons of the thalamic parafasicular nucleus were intracellularly labeled by iontophoretic injection of HRP and analyzed under the light microscope. At the end of the physiological experiment, rats were perfused with a mixture of 1% formaldehyde and 2% glutaraldehyde. The brains were sectioned at 50 um in sagittal plane and reacted with diaminobenzidine procedure. More than half of these neurons were antidromically activated following striatal stimulation. The somata of PF neurons were spine free and polygonal or oval in shape with the shortest diameter being 6-11 um and the longest diameter being 7.5-16 um. Three to five primary dendrites arose from the soma. These dendrites tapered slightly and divided into relatively thin secondary branches within 50 um from the soma. Most of the secondary dendrites divided further into thin branches. The dendritic branching ratio (i.e. dendritic tips/primary dendrites) ranged from 4-7. The proximal dendrites were generally smooth but the distal dendrites were sparsely covered with spines. Varicosities were frequently observed along the distal portion of the dendrites. The dendritic fields were oval in shape having slightly longer rostral-caudal axes (up to 800 um) than the ventro-dorsal or medial-lateral axes (up to 600 um). Some dendrites extended into neighbouring thalamic nuclei. The axons usually emerged from the soma. The axons of all the neurons analyzed were traced outside of Pf indicating that they were projection neurons. Only one axon was traced ventrally into the zona incerta. All the other axons were traced rostrally toward the internal capsule. In general, no axon collaterals were found either in the PF or other thalamic nuclei except one axon emitted a collateral into the reticular thalamic nucleus. These observations indicate that rat PF may be comprised of one type of projection neuron with large overlapping dendritic fields. Most PF neurons send their axons rostrally to the striatum with very little collateralization within the PF nor in the thalamic reticular nucleus. (We thank Dr. K. D. Phelan for help with some experiments. Supported by NIH Grant NS20702).

205.11 A GABAergic RESPONSE IN STRIATAL SLICE PREPARATION. T.Kita, H. Kita and S. T. Kitai. Div. of Neurosci., Dept. of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163

It is well demonstrated that the striatal medium spiny projection neurons have extensive intrinsic axon collaterals. The function of these collaterals was suggested to be inhibitory and mediated by GABA. This study was intended to reveal the latency, duration and reversal potential of the GABAergic inhibitory responses using a striatal slice preparation. Rat striatal slices about 400 um thick were sectioned using a Vibratome. A slice placed in a recording chamber was continuously superfused by oxygenated Ringer solution. Glass micropipettes filled with 2M K-methylsulfate and 0.1M QX-314 were used for intracellular recordings. When a neuron was penetrated, QX-314 was injected through the recording electrode to block generation of action potentials. In this preparation postsynaptic responses could be tested with artificial depolarization without being confounded by the generation of action potentials. Local stimulation applied through a bipolar electrode at 0.5-1mm from the recording electrode, evoked depolarizing postsynaptic responses in striatal neurons at resting membrane potential. When the neuron was depolarized, local stimulation evoked depolarizing potentials followed by hyperpolarizing potentials. Further increases in depolarizing current intensity resulted in local stimulation evoking only hyperpolarization. The latency of hyperpolarizing responses under these conditions was about 3.8 ms (n=30) and was not altered by changes in stimulus intensity. These observations suggested that the response was monosynaptic. The maximum duration of the hyperpolarizing response to strong local stimulation was about 130ms. The hyperpolarizing response was blocked by the application of bicuculline or picrotoxin. Combined intracellular Cl and QX-314 injections produced changes in the response pattern to the local stimulation. The initial depolarizing response was followed by another relatively large depolarization which no longer could be reversed by the intensity of depolarizing current previously used to reveal hyperpolarization. Those results indicated that in the striatal slice there is monosynaptic Cl-mediated GABAergic response with a maximum duration of 130msec and having a reversal potential of -60mV. This response is probably mediated by axon collaterals of medium spiny neurons since there are no reported monosynaptic extrinsic inhibitory inputs to the striatum. (QX-314 was generously donated from the Astra Pharmaceutical Products, Inc. (Supported by NIH Grant NS20702.)

205.12 ELECTRICAL MEMBRANE PROPERTIES OF RAT SUBSTANTIA NIGRA PARS COMPACTA (SNc) NEURONS: INTRACELLULAR STUDY IN IN VITRO SLICE PREPARATIONS. H. Kita, T. Kita and S. T. Kitai. Div. of Neurosci., Dept. of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163

Rat brain tissue containing the SNc was sectioned on a Vibratome (350-450 um) in the parasagittal or cronal planes. Slices were placed in a recording chamber and continuously superfused by oxygenated Ringer solution. Intracellular recording electrodes were visually placed in SNc. Some of the recorded neurons were intracellularly stained by iontophoretic injections of HRP. The input resistance of SNc neurons at resting membrane potential ranged from 70 to 250 Mohm. Intracellular hyperpolarizing current injections revealed the existence of anomolous rectification. Following the off set of hyperpolarizing current pulses, two distinct membrane responses were observed. One response, probably due to early K conductance, was characterized by a slow ramp-shaped recovery of membrane potential from the hyperpolarization. The other was a rebound depolarization which usually followed the ramp-shaped recovery. The amplitude of this rebound depolarization was diminished by superfusing the preparations with Ca free high Mg medium. Depolarizing current pulse injections generated slow and small amplitude depolarizing responses which could lead to the generation of action potentials. The slow depolarizing response was diminished by a substitution of Ca to Mg in the superfusing medium. The response after the offset of the depolarizing current pulse was a long-lasting hyperpolarization. Similar long-lasting hyperpolarizations were observed after an action potential. The amplitude of these long-lasting hyperpolarizations was decreased or abolished by either intracellular EGTA injections or superfusing Ca free medium. Injection of depolarizing current generated rhythmic discharges in SNc neurons. Injections of higher intensity current decreased the inter-spike-intervals and the spike amplitudes. The decrease of the spike amplitude occurred abruptly in stepwise fashion as the current intensity was increased. These rhythmic discharges were abolished by TTX. In conclusion, rat SNc neurons have rather high input resistance and therefore even small synaptic inputs would produce larger effects on the neuron activity. Our observations also suggest that Ca-conductance is responsible for rebound depolarization and Ca-dependent K-conductance responsible for long-lasting spike hyperpolarization. Therefore, Ca- and K-conductance would be likely to have important roles in controlling rhythmic discharge of SNc neurons. (Supported by NIH GrNS20702)

205.13 CAUDATE EFFERENTS IN PERINATAL RATS ORIGINATE PRIMARILY FROM PATCHES RECEIVING DOPAMINERGIC INPUT. G. Fishell* and D. van der Kooy (Spon: Y. Israel). Department of Anatomy, University of Toronto, Toronto, Canada M5S 1A8

Specific neurotransmitters, receptors and afferent neuronal fibers occur in patches in the caudate putamen. During perinatal development, opiate receptor patches occur in aggregations that exactly match the distribution of patches of dopamine containing nerve terminals from the substantia nigra. We now report that dopamine patches also exactly match the distribution of caudate cells sending prominent reciprocal efferent connections to the perinatal substantia nigra. In the adult rat both dopamine fibers and striatal cells projecting to the nigra become more diffusely distributed throughout the caudate. Injections (.1-.2 ul) of the fluorescent retrograde axonal tracer propidium iodide (P.I.) were made into the substantia nigra of 2 day old rat pups. After a 48 hour survival period sections through the caudate-putamen were processed to visualize endogenous dopamine (SPG method). The striking observation was that groups of striatal cells showing heavy retrograde labeling with P.I. occurred in patches in exactly the areas of the perinatal caudate-putamen innervated by substantia nigra dopaminergic fibers. Striatal cell bodies outside dopamine patches were labeled more faintly with retrograde P.I. fluorescence. The heavier retrograde labeling of striatal cells in the dopamine patches might be due to their elaborate axonal terminations in the substantia nigra, their more efficient transport mechanisms or less efficient metabolism of tracer. Preliminary observations suggest that the distribution of dopamine matched striatal efferent cells regardless of whether the perinatal P.I. injections were made laterally in the substantia nigra or medially in the ventral tegmental area. The match of dopamine terminals and striatal cells with heavy retrograde labeling is not an artifact; the contralateral caudate contained patches of dopamine fluorescence without P.I. retrograde labeling and some ipsilateral areas where the SPG treatment failed to visualize dopamine still showed patches of retrograde labeling under the appropriate specific filter. These results suggest that an inductive developmental relationship may exist between the caudate-putamen and the substantia nigra-ventral tegmental area. It is not clear which of the nigrostriatal or striatonigral pathways is the putative inductor of the other, because the time course of development of the striatonigral pathway has not been investigated.

205.14 MECHANISMS UNDERLYING THE DEVELOPMENT AND MAINTENANCE OF A PATCHY STRIATAL ORGANIZATION. D. van der Kooy, A.J. Lanca* and B.E. Kolb, Dept. Anatomy, Univ. Toronto, Med. Sch., Toronto, Canada M5S 1A8.

A patchy organization of the rat caudate-putamen can be seen to develop perinatally. Striatal opiate receptor patches develop so that they exactly match both the perinatal distribution of patches of dopamine fibers from the substantia nigra and the areas of less neuronal cell density in the perinatal striatum. We asked whether intrinsic or extrinsic striatal factors were responsible for the development and stable maintenance of the patchy striatal organization. To test the importance of intrinsic factors, we transplanted pieces of fetal (E12-E19) rat caudate into the caudal cortex of young adult rat hosts. Opiate receptors studied autoradiographically using 1nM 3H-etorphine were present diffusely in the transplants, except that areas of less neuronal density had somewhat higher concentrations of opiate receptors. However, the very dense patches of opiate receptors that normally develop in the caudate-putamen did not develop. To test the importance of extrinsic factors in patch development we made early postnatal lesions of two major extrinsic neuronal connections of the striatum. Knife cut lesions caudal to the striatum in the early postnatal period resulted in a greater than 60% decrease in the percentage of the area of the striatum covered with opiate receptor patches in the young adult. On the other hand, complete decortication at postnatal day 1 increased the density of opiate receptor patches by 50% as assessed in young adult rats. To test the mature stability of opiate receptor patches we injected naloxazine (5 ug in .5 ul; an irreversible opiate receptor antagonist) into the striatum of adult rats. As new opiate receptors were synthesized they appeared in a normal patchy pattern in the striatum. Thus striatal patches appear to be a stable and maintained structure in the adult rat. We suggest at least 2 processes are involved in the development and stabilization of striatal transplants, an initial intrinsic process in patch formation involves a local decrease in density of neuronal cell bodies (possibly due to neurite outgrowth of certain intrinsic striatal cells). However, as evidenced by the caudal knife cut data, a secondary extrinsic process, possibly involving neuronal connections with the substantia nigra is crucial to the development and stabilization of the large numbers of the high opiate receptor density patches seen in the adult striatum.

205.15 THE DOPAMINE-CONTAINING INNERVATION OF THE CAUDOPUTAMEN IS PRESENT AT BIRTH IN THE WEAVER MUTANT AND FORMS ISLANDS, BUT FAILS TO DEVELOP NORMALLY. S.Roffler-Tarlov and A.M.Graybiel, Depts. Neurol. and Anat., Tufts Univ. Sch. Med., Boston, MA 02111 & Mass. Inst. Tech., E-25, Cambridge, MA 02139.

We reported recently that the autosomal recessive mutation carried by the mutant mouse weaver produces differential effects on the DA-containing innervation of the limbic and the non-limbic striatum. (Nature, 307, 63 1984). Specifically, the dorsolateral caudoputamen (CP), a target of the nigrostriatal system, is severely depleted of DA (-70%) whereas DA is entirely conserved in the n. accumbens (NAc), a target of the mesolimbic system. The olfactory tubercle (OT) shows a 30% reduction in DA.

We now report that in weaver neonates, the DA-containing innervation of the CP is characterized by normal DA concentrations and by a normal anatomical arrangement into DA "islands". Subsequently, however, the weaver disease is expressed in the CP as a failure of this early DA-containing innervation to develop normally. We compared the content of DA extracted from three divisions of the striatum (CP, NAc and OT) and from the midbrain of weaver and control littermate pups bred on a C57BL6/CBA background. Catecholamines were extracted from tissues dissected from serial brain slices and were separated and measured using HPLC followed by electrochemical detection. Values were expressed as pmoles DA/mg protein (mean $\pm$ SEM). DA was not reduced in any region of 7 day-old weaver mice. The values were: for CP, 91 $\pm$ 3 in controls, 105 $\pm$ 4 in weavers; for NAc, 220 $\pm$ 13 in controls and 210 $\pm$ 15 in weavers; and for OT, 175 $\pm$ 12 in controls and 180 $\pm$ 25 in weavers. The DA content in CP of control animals 5 days older had increased to 160 $\pm$ 7 but the DA content of weaver CP was 130 $\pm$ 9. The DA content of both NAc and OT was normal in these 11-day old weaver animals; DA in midbrain was reduced (17 $\pm$ 1 in control and 10 $\pm$ 1 in weaver). The DA content of CP failed to increase after the second postnatal week: in the controls it had doubled by 30 days.

The pattern of catecholamine innervation of the developing striatum was studied in 8 and 11 day-olds using tyrosine hydroxylase (TH) immunohistochemistry. Patches of TH-like immunoreactivity, characteristic of the normal "islandic" pattern of innervation in neonatal CP, were present in the developing CP of weavers and controls at both ages. In the midbrain, there was no dramatic loss of cells stained for TH. We conclude that the weaver defect can be detected biochemically at a time when DA islands are still present.

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205.16 OVERLAPPING DISTRIBUTIONS OF GABAERGIC AND CHOLINERGIC NEURONS IN THE DIAGONAL BAND OF THE RAT. H.R. Brashear*[1], L. Zaborszky[1], D. Schmechel*[2] and L. Heimer[1]. (SPON: F.E. Dreifuss). 1. Dept. of Neurol., UVA Med. Ctr., Charlottesville VA, 2. Duke Univ. Med. Ctr., Durham, N.C.

GABAergic neurons are coextensive with the cholinergic neurons within the medial septal nucleus-diagonal band (DB) complex. To test for co-localization of these two transmitters we used several methods with antibodies to glutamate decarboxylase and choline acetyltransferase: immunostaining of serial 5 µm frozen sections, sequential staining of 20 µm sections with peroxidase-antiperoxidase techniques and double immunofluorescence staining of 20 µm sections.

Although the two types of neurons could not be distinguished on the basis of morphological features, they were characterized by distinctive, but overlapping, distributions in the diagonal band. GAD-positive cells were scattered diffusely through the nucleus of the vertical limb of the DB (nVLDB), while ChAT-positive neurons tended to be localized medially within the nVLDB and to be separated into two groups corresponding to the dorsal and ventral aspects of the nucleus. In the rostral parts of the nucleus of the horizontal limb of the DB (nHLDB), the ChAT-positive cells tended to be located medial to GAD-positive cells, whereas in more caudal sections, they spread dorsally through the lateral hypothalamic area to become continuous with other large cholinergic neurons of the basal forebrain system. The large majority of GAD-positive neurons, on the other hand, remained in a more ventral and lateral position within the nHLDB. The cholinergic neurons were estimated to be about two to three times more numerous than identified GABAergic neurons. Less than 2% of the ChAT-positive neurons were double labeled. However, since we cannot rule out the possibility of artifactual double-labeling because of minimal cross-reactivity between the secondary antibodies used, the question of co-existence of the two transmitters in a minority of DB neurons remains open.

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205.17 NEUROTOXIC EFFECTS OF THE MEPERIDINE ANALOGUE N-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (NMPTP) ON IMMUNOREACTIVE TYROSINE HYDROXYLASE IN RAT SUBSTANTIA NIGRA. L. L. Vacca, R. Ikeda*, M. E. Melnick and K. Shellenberger, Depts. Of Anatomy, Physical Therapy Education and Pharmacology, Univ. Kansas Med. Ctr., Kansas City, KS 66103.

Human abuse of a meperidine-analog contaminated with NMPTP causes chronic Parkinson's disease within 1-2 weeks (Davis et al., Psychiat. Res., 1:249, 1979; Langston et al., Science, 219: 979, 1983). The contaminant produced a selective damage of dopamine (DA) neurons in substantia nigra (SN) as detected by chemical and histological analyses. Additional data have been obtained in monkeys (Burns et al., Proc. Nat. Acad. Sci., 80: 4546, 1983). In mice, NMPTP is reported to produce a rapid and long-lasting reduction of striatal DA, and in striatum and frontal cortex NMPTP induced a long-term reduction of noradrenaline (Hallman et al., Eur. J. Pharmac., 97:133, 1984).

In our laboratory, we injected NMPTP in doses of 3-30 mg/kg into adult male Spargue Dawley rats (400-500 gm) for periods ranging between one to 10 days. Immediately after treatment, some rats exhibited clonic seizures; most rats maintained a flattened quiescent posture. A series of behavioral tests were applied to evaluate alterations in motor activity possibly related to basal ganglia function (residential exploratory activity and symmetry of walking patterns). The rats were then anesthetized, perfused with buffered formalin, and their brains dissected and prepared for the immunocytochemical visualization of tyrosine hydroxylase (TH), a marker for DA neurons. Preliminary results indicate that rats treated with 30 mg/kg doses of NMPTP have reduced amounts of immunoreactive TH within SN neurons (perikarya and processes) as determined by the end-point of immunostaining and by densitometry. Morphometric studies are underway to determine whether the number of perikarya is also reduced. These data indicate that the rat substantia nigra is vulnerable to damage by NMPTP.

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205.18 MURINE MODEL OF MPTP-INDUCED PARKINSONISM:HISTOPATHOLOGY. A. Hess, D. Yamasaki, A. Bretschneider*, I. Meadows*, P. Adamo*, R.E. Heikkila and R.C. Duvoisin. Departments of Anatomy and Neurology, UMDNJ, Rutgers Medical School, Piscataway, NJ 08854.

The inadvertent injection of MPTP (1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine) in several young people has resulted in marked signs of parkinsonism whose symptoms were alleviated by l-dopa (Langston et al, 1983). These incidents gave rise to the idea that MPTP administration to animals might lead to the production of parkinsonism. Parkinsonian features have been produced in rhesus (Burns et al, 1983) and squirrel (Langston et al, 1984) monkeys. The production of parkinsonism in a rodent or other small animal would be advantageous in making such material more accessible and less expensive and should serve to instigate more extensive study of Parkinson's disease.

We believe that we have produced a murine model of parkinsonism. Injections of MPTP have resulted in decreases in dopamine content in the striatum, deficiencies of dopamine uptake in the striatum, neuronal cell loss in the pars compacta of the substantia nigra, terminal fiber degeneration in the caudate nucleus, and tonic behavior in some mice--all of these being characteristic of the chemical, histopathological and behavioral features of Parkinson's disease in man.

Male Swiss Webster mice were injected intraperitoneally with 30mg/kg MPTP in distilled water with the pH adjusted to 8.5 with dilute hydrochloric acid. Five to 10 injections of MPTP resulted in pronounced decrements (67-80%) in levels of dopamine and its metabolites in the striatum; nucleus accumbens and hypothalamus were unaffected; neostriatal levels of serotonin and its metabolites were unaffected.

Histopathological studies of control and MPTP treated mice were performed on littermates of the above animals exhibiting dopamine deficiencies. Cresyl violet staining of paraffin and frozen sections revealed a marked bilateral loss of neurons in the pars compacta of the substantia nigra. Retrograde transport of horseradish peroxidase (HRP) after large injections into the caudate nucleus unilaterally resulted in a striking demonstration of the decrease in number of pars compacta neurons after MPTP treatment.

C57 Bl mice were even more susceptible to MPTP than Swiss Webster. Dopamine losses were greater after fewer injections of MPTP. Selective silver impregnation (Fink-Heimer) revealed degenerating terminals in the caudate nucleus in these black mice after only 3 injections of MPTP.

205.19 NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE-DIAPHORASE (NADPH-d) HISTOCHEMISTRY OF THE HUMAN CAUDATE NUCLEUS. R.J. Ferrante* and N.W. Kowall. Dept. of Pathology and Neurology, Massachusetts General Hospital, Boston, MA 02114.

NADPH-d reactive neurons in the rat striatum represent a subset of cells that contain both somatostatin and neuropeptide Y-like immunoreactivity (Vincent, S.R. et. al., J. Comp. Neurol. 217:252-263,1983). We have modified a direct method of NADPH-d histochemistry in order to assess the morphometry and distribution of diaphorase positive cells in formalin fixed human caudate nucleus. This NADPH-d method allows rapid and reliable visualization of neurons in detail, delineating their dendritic and axonal arbors.

Seven blocks of human caudate nucleus were obtained 8-16 hours postmortem and immersed in 10% neutral buffered formalin at 4°C. After 48 hours fixation, the tissue was frozen in isopentane cooled to -70°C with liquid nitrogen. Cryostat sections were cut at -20°C and stained for NADPH-d activity by incubating free floating 50 µm sections in 10 ml. Tris-HCl buffer solution (pH 7.4) containing 10 mg. NADPH and 4 mg. Nitro Blue Tetrazolium (NBT) salt at 37.5°C for 1 hour. The NBT was reduced in the presence of NADPH to an insoluble blue end product, formazan, marking positive cells.

Stained cells stood out on a clear background. They were distributed in a strial pattern leaving patches which were devoid of positive reaction. Morphologically, the stained cells resembled aspiny neurons, as previously described with cytochemical techniques for neuropeptide Y and somatostatin-like immunoreactivity (Vincent, S.R. and Johansson O., J. Comp. Neurol. 217:264-270, 1983).The cell soma were medium to large in size and pyramidal, spheroidal or fusiform in shape. Some nuclei were eccentric with invaginations. The dendritic arborizations were either multipolar with multiple branching or bipolar with sparse elongate ramifications.

This method should be useful for the examination of human brain tissue under pathological conditions such as Huntington's disease (in which both neuropeptide Y and somatostatin are said to be preserved). The osmiophilic properties of the formazan end product will allow the ultrastructure of these cells to be studied. Simultaneous histochemistry for both diaphorase and cholinesterase activity as well as confirmation of immunocytochemical co-localization of somatostatin and neuropeptide Y with diaphorase in human striatum should also be possible.

205.20 CHANGES IN THE DENDRITE MORPHOLOGY OF MEDIUM-SIZE NEOSTRIATAL SPINY NEURONS IN HUNTINGTON'S DISEASE. M.DiFiglia, G.A. Graveland and R.S. Williams. Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114.

The neostriatum was examined in Golgi impregnations of Huntington's disease (HD; N=10) and control brains. Controls consisted of age-matched normals (N=4) and a group with other neurologic disorders (N=5), including one each of Wilson's disease, Parkinson's disease and massive cortical infarct, and two cases of schizophrenia. Results showed that in HD, medium-size neurons found mostly in the caudate nucleus were severely degenerated and could not be classified. Such cells were characterized by a few thin, truncated dendrites with irregular contours and few or no spines. Cell bodies, primary dendrites and axon initial segments were also irregular in contour with some focal swellings. Other neurons in the caudate and putamen, were less atrophic and retained the characteristic features of medium-size spiny cells based on dendrite branching and spine distribution. However, they exhibited changes in dendrite morphology consisting of recurved endings within the distal one-eigth to one-third of their dendritic fields. The bending produced a change of 90° or more in the orientation of dendrites, which sometimes exhibited spiral or S-shaped configurations as well. Quantitative studies showed that neostriatal spiny cells with recurved dendrites comprised the majority of neurons in the HD cases (63-100% of total neurons sampled), occurred infrequently in normals (0-13%) and in some of the disease controls (Parkinson's 9%; Schizophrenia, 9%) and appeared with somewhat increased frequency in Wilson's disease (41%) and in the neostriatum denervated by cortical infarction (24%). The recurved dendrites of HD spiny cells appeared to represent new growth. Quantitative studies confirmed that the recurved dendrites in HD were longer than uncurved dendrites from the same neurons, or the longest uncurved dendrites of normal controls. Other changes observed included a marked reduction in spine density in most neurons but a paradoxical increase in spines in the dendrite segments of some cells (6-13% of total HD neurons sampled). Pathologic changes were rarely observed in other types of neostriatal neurons identified as belonging to the medium- and large-size aspiny types. Results suggest that in the HD neostriatum medium-size spiny neurons are preferentially affected and exhibit both degenerative and regenerative changes. Supported by NIH Grant NS 16367 (MD) and MH34079 (RSW).

RESPIRATORY REGULATION

206.1 ANATOMY AND CENTRAL PROJECTIONS OF THE TYMPANIC PLEXUS IN THE CAT. P.J. Gannon and A.R. Eden, Department of Otolaryngology, Mount Sinai School of Medicine, New York, New York 10029.

Little is known about the component nerves and discrete functions of the middle ear tympanic plexus (TP) in the cat. This study was designed to investigate the anatomy and central projections of this region.

Adult cats were anesthetized with ketamine hydrochloride (30mg/kg). The middle ear was exposed through the tympanic bulla in seven animals. Gelfoam impregnated with horseradish peroxidase (HRP, 30% solution in saline, type VI [Sigma]) was applied directly to the transected plexus nerves on the promontory of the middle ear. After a 24-48 hour survival, 40µ serial transverse brainstem sections were reacted by the tetramethylbenzidine (TMB) blue reaction process. The contralateral middle ears and bullae of the perfused animals were flooded with osmium tetroxide (2% solution in 0.1M phosphate buffer, pH 7.4) to selectively stain the barely visible nerves of the tympanic plexus.

Anatomically, the plexus was noted to be much more extensive than in macaques (rhesus, cynomolgus) or humans. Unlike primates, however, most of the TP nerves course under a bony ridge separating the air-containing bulla from the middle ear, and cross the bulla to the jugular foramen.

HRP-labeled neurons were observed in the ipsilateral superior and inferior salivary nuclei. These labeled salivary neurons demonstrate the passage of preganglionic parasympathetic secretomotor fibers through the tympanic plexus as in the human.

Extensive HRP-labeled terminal fields were observed in that part of the ipsilateral solitary tract nucleus (NTS) known to receive chemo- and baroreceptor input from the lower respiratory tract. These projections suggest that a component of the TP may monitor pressure and/or gas composition in the middle ear.

(Supported in part by NIH Grant NS 19179)

206.2 COMPARISON OF EFFECTS OF SUPERIOR LARYNGEAL (SL) NERVE AND ROSTRAL PONTINE (NUCLEUS PARABRACHIALIS MEDIALIS: NPBM) STIMULATION ON DORSAL RESPIRATORY GROUP (DRG) INSPIRATORY (I) NEURON AND PHRENIC DISCHARGES. A.L. Sica. D.F. Donnelly*, M.I. Cohen and H. Zhang*. Dept. Physiol., Albert Einstein Coll. Med., Bronx, N. Y. 10461.

In decerebrate, paralyzed cats ventilated with a cycle-triggered pump, the responses of DRG I neurons of the medulla and of the whole phrenic nerve to NPBM and SL stimulation, both of which produce inhibition of I activity, were compared. Single-shock stimulation (.05 msec duration, 60-120 µA for SL, 400-800 µA for NPBM) was ipsilateral to the unit recorded and was delivered during the second half of the I phase. For each type of stimulation, current intensity was adjusted to a level slightly higher than the threshold for eliciting an inhibition of phrenic activity; with NPBM stimulation, two shocks were sometimes required to achieve this effect. SL stimulation usually produced an excitation (5 msec latency) of the contralateral phrenic, followed by a bilateral phrenic inhibition (8 msec latency, 28 msec duration). Neurons (n = 14) responded with an inhibition (6 msec latency, 28 msec duration); 8/14 neurons had an excitation (3 msec latency) which preceded the inhibition. With NPBM stimulation, a short latency (5 msec) bilateral phrenic inhibition, lasting 15-17 msec, was observed. Neurons (n = 11) responded with inhibition; the average latency and duration did not differ significantly from those of phrenic inhibition. In 4 cases, a short latency (2 msec) excitation preceded the inhibition. Thus, the latencies of DRG I neuron responses to SL stimulation were less than those of phrenic responses, whereas the latencies of their responses to NPBM stimulation were not less than those of phrenic responses. These results suggest that the effects of SL stimulation on phrenic discharge are mediated by DRG neurons, while NPBM effects are mediated by some other population, perhaps the ventral respiratory group. (Supported by USPHS Grants HL-27300 and HL-07060.)

206.3 ROLE OF THE DORSAL RESPIRATORY GROUP (DRG) IN PROCESSING VAGAL AND SUPERIOR LARYNGEAL NERVE AFFERENT INPUT IN CAT. D.R. McCrimmon, D.F. Speck & J.L. Feldman, Depts. of Physiology & Anesthesia, Northwestern U., Chicago, IL 60611.

We investigated the effect of uni- or bi-lateral destruction of the ventrolateral nucleus of the tractus solitarius (i.e. DRG) on the processing of afferent input from the vagus (X) and superior laryngeal (SL) nerves. Experiments were conducted on anesthetized, vagotomized, paralyzed and artificially ventilated cats. We verified that 1) single electrical pulses (5-30 uA, 0.1 ms duration) to the SL nerve during inspiration elicit a short onset latency (4-6 ms) excitation of the contralateral (only) phrenic nerve followed by a bilateral inhibition, 2) at higher currents (50-150 uA) a post-inhibitory excitation is observed, and 3) stimulus trains delivered to the X nerve (20-60 uA, 100 Hz, 0.1 ms pulse duration; intensities chosen to maximize activation of pulmonary stretch receptors) throughout inspiration produce a current dependent shortening of inspiratory duration (TI). We recorded and lesioned in the DRG with a linear array of 2 to 4 tungsten microelectrodes (tip separation of 0.7 to 1 mm). When DRG respiratory neurons were recorded on an electrode it was moved to the approximate center of this activity and a lesion was made by passing current (20 uA, electrode negative for 60 sec). If respiratory neurons were recorded on an adjacent electrode, then lesions were made between these electrodes (20 uA for 60 sec each polarity). We moved the array in 500 um steps (in the horizontal plane) and iterated the above procedure until respiratory neurons were no longer detected. Lesions were located ventral and lateral to the tractus solitarius and extended from about 1 mm caudal to 2 mm rostral to the obex. Uni- or bi-lateral DRG destruction (verified to be at least 80% complete) abolished the short latency phrenic nerve excitation to SL nerve stimulation; the longer latency bilateral phrenic nerve inhibition and subsequent excitation were unaffected. The relative shortening of TI in response to X nerve stimulation of a given intensity was the same before and after lesioning. We conclude: 1) the DRG is an obligatory component in the short latency phrenic nerve excitation elicited by SL nerve stimulation, and 2) neuronal pathways exclusive of the DRG are sufficient to produce inspiratory termination in response to activation of pulmonary stretch receptor afferents. (Supported by NIH grant HL-23820, D.R.M. is a Parker B. Francis Fellow of the Puritan-Bennett Foundation).

206.4 CARDIORESPIRATORY RESPONSE TO SUPERIOR LARYNGEAL NERVE STIMULATION BEFORE AND FOLLOWING ANESTHESIA. D.F. Donnelly and G.G. Haddad. Dept. Pediatrics, Columbia University, New York, NY 10032.

Superior laryngeal nerve (SLN) stimulation produces asphyxial death in anesthetized young piglets (Lee et al, AJP 233:R30,1977). However, previous work in this laboratory showed that unanesthetized piglets have no depression of cardiovascular function during SLN stimulation despite a profound depression of breathing. The purpose of these experiments was to reconcile these observations by quantitating the effects of laryngeal stimulation at a given level before and following anesthesia.

Both SLNs of 6 piglets (age 6-37 days) were ligated, cut and placed in cuff electrodes one to two days before the experiment. During each experiment we measured ventilation (barometric plethysmograph), arterial pressure and blood gases (femoral cannula) and recorded EEG and EKG. Both SLN were stimulated for 30 minutes at 2-4x threshold for respiratory depression (10 Hz, biphasic 40ms, 0.8+0.3 v.). Blood gases were drawn before and at 5 and 15 minutes into the stimulus period. Following recovery from stimulation, animals were anesthetized with pentobarbital (25-35 mg/kg i.a.) and the SLNs were again stimulated at the same intensity. Results from 4 piglets <2 weeks old were as follows:

	Control	@5 min	@15 min
PaO_2 before anesthesia	112+3	89+12	92+8
PaO_2 following anesthesia	119+7	22+7	26+9 *
$PaCO_2$ before anesthesia	33+1	44+1	44+2
$PaCO_2$ following anesthesia	44+1	66+4	74+7 *

(*2/4 animals B.P. 10 mmHg at which time the stimulus was removed and CPR initiated.)

Unanesthetized piglets continued breathing during the stimulation period with breaths closely associated with opening of the eyes and body movement. Lightly anesthetized piglets responded with a profound apnea (>1 min) and suffered cardiorespiratory collapse or breathed occasionally in a gasping pattern. These gasps sustained cardiovascular function to a remarkable degree. This profound respiratory depression was never seen in older piglets (>3 weeks) either before or following anesthesia. We conclude that during laryngeal reflex activation, arousal plays a vital role in sustaining breathing in young animals and that anesthesia can blunt this protective mechanism.

206.5 RELATIVE CONTRIBUTION OF PULMONARY STRETCH RECEPTOR (PSR) INPUT FROM IPSI- AND CONTRALATERAL VAGI ONTO DORSAL RESPIRATORY GROUP (DRG) R-BETA NEURONS. L. Kubin* and R.O. Davies. Dept. of Animal Biology and Cardiovascular-Pulmonary Div., Univ. of Penna., Phila., PA 19104

DRG R-beta neurons receive a strong excitatory input from PSR afferents. Recently, two new observations on PSR input to DRG neurons in the cat were reported. Donoghue et al. (J. Physiol. (London) 322:353-363, 1982) showed that some PSR central terminals reach the ventro-lateral nucleus of the solitary tract (NTS) where DRG cells are located; no projection has been traced to the contralateral DRG. In another study, Averill et al. (Physiologist 26:A-45, 1983) demonstrated that some PSR afferents excite monosynaptically R-beta neurons of the ipsilateral DRG. However, anatomic studies of Kalia and Mesulam (J. Comp. Neurol. 193:435-464, 1980) have indicated that afferents from the main bronchus project to both the ipsi- and contralateral NTS. In the present study, we determined whether, in addition to an ipsilateral input, DRG neurons receive a relay from PSR in the contralateral vagus.

In decerebrate cats, we recorded from individual DRG neurons and identified them as R-beta by a "no inflation" test. Then the transmission in myelinated fibers of the ipsilateral vagus was reversibly blocked by application of a constant de- or hyperpolarizing current on the nerve. The completeness of the block was assessed by recording the evoked vagal compound action potential. In all animals, the Hering-Breuer inspiratory inhibitory reflex could be elicited both with and without the unilateral vagal blockage.

In 8 out of 12 R-beta neurons studied to date, ipsilateral vagal blockade abolished their excitatory response to PSR input. In another 3 cells, a strongly reduced PSR input persisted during the block. In one cell, the PSR input originated almost exclusively from contralateral PSR afferents; this was tested by ipsilateral blockade and a subsequent section of the contralateral vagus nerve.

We conclude that most of the R-beta neurons receive their PSR input only from the ipsilateral vagus. However, a smaller population has a bilateral input. In a further extension of this study we will attempt to determine whether there are other differences between properties of those R-beta cells with an ipsilateral and those with a bilateral convergence of PSR input. (Supported by USPHS Grant HL-08805)

206.6 AVERAGING OF MEMBRANE POTENTIAL OF VENTRAL RESPIRATORY GROUP NEURONS TRIGGERED BY RETRO-FACIAL RESPIRATORY UNIT SPIKES. J.E. Remmers, R. Takeda*, S. Schultz*, and A. Haji*. Dept. of Int. Med., UTMB, Galveston, Tx. 77550-2778.

Early inspiratory (EI) neurons of the retro-facial nucleus (RFN) project contralaterally to the vicinity of the bulbar ventral respiratory group (VRG) (Bianchi, A.L. and J.C. Barillot, *Neurosci. Let.*, 31: 227, 1982). Post-inspiratory(PI) neurons of the VRG receive prominent inhibitory post-synaptic potentials (IPSPs) during inspiration, and their membrane is often maximally polarized early in inspiration. We speculate that PI neurons receive IPSPs from axons of EI neurons located in the contralateral RFN. To examine this possibility, we simultaneously recorded in decerebrate cats extracellular action potentials of respiratory units in the RFN and membrane potential (MP) of VRG respiratory using glass micropipettes filled with 3 M KCl. The animals were paralyzed and ventilated by a phrenic driven servo-respirator. All neurons were tested for peripheral axons by stimulating the ipsilateral vagus and superior laryngeal nerves, as well as the spinal cord. Pre- and post-spike histograms of MP of the VRG neuron were calculated using the AP of the RFN unit as a trigger. Stable simultaneous recordings of MP and of Aps were obtained in 32 instances. In addition to EI and PI neurons, augmenting inspiratory (AI) and expiratory (AE) neurons were recorded in both locations. Averaged MP waveforms possibly indicative of short latency of IPSP's or EPSP's following the RFN spike trigger were observed in 17-82 pairs. The distribution of these correlations amongst 4 categories of respiratory neurons is shown in the following matrix.

VRG MP	RFN SPIKE EI	RFN SPIKE AI	RFN SPIKE PI	RFN SPIKE AE	Category Totals
EI	1/11	0/3	0/1	2/4	3/19
AI	1/10	0/6	0/1	0/5	1/22
PI	8/13	2/11	0/4	1/2	11/30
AE	2/10	0/1	--	--	2/11

The high percentage of correlations between EI spike and PI membrane potential is consistent with the original speculation. In these instances, a hyperpolarizing wave of 8-10msec duration followed the RFN spike. Reversal of this wave after Cl^- injection was observed, suggesting that the wave results from the arrival of IPSPs at the VRG neuron shortly after the RFN spike. (Supported by NHLBI Grant HL-27520).

206.7 PROLONGATION OF POST INSPIRATORY INSPIRATORY ACTIVITY (PIIA) DURING AUGMENTED BREATHS. J. Mitra, N.R. Prabhakar,* and N.S. Cherniack*. Dept. of Medicine, Case Western Reserve Univ., Cleveland, OH 44106.

The phase I of expiration with decaying post inspiratory discharge of the inspiratory muscles is called "post inspiratory inspiratory activity" (PIIA)(Richter, D.W. and Ballantyne, D., Central Neurone Environment, Springer Verlag 169-174, 1983). We analyzed the duration of the PIIA in cats anesthetized with pentobarbital sodium (n = 8) or chloralose (n = 2), during different trajectories of the phrenic activity. Augmented breaths (AB) were characterized by a biphasic shape of the phrenic trajectory (Cherniack et al., Acta Physiol. Scand. 111:349-360, 1981). Spontaneous AB's were observed in vagotomized, carotid sinus denervated cats, ventilated with 7% CO_2 in O_2. The duration of PIIA (T_{E_1}) in the control breaths was 0.24 ± 0.02 s (mean ± SEM), while in AB's it was 0.54 ± 0.06 s (n = 14), which is a 225% increase. Similar increase in T_{E_1} (PIIA) was also seen during AB's in spontaneous breathing animals with intact vagi and sinus nerves and also animals breathing 100% O_2. However, T_{E_2} (the duration of expiration without PIIA) increased from 0.79 ± 0.05 to 0.92 ± 0.05 s, an increase of 116.5%. No or almost negligible PIIA was seen with apneustic type of phrenic activity. PIIA was decreased or even absent immediately after the denervation of carotid sinus nerves; however, after 5 to 10 min PIIA reappeared with duration similar to the predenervated controls, suggesting CSN are not essential for the presence of PIIA. PIIA is present in both types of anesthesia used in this study. The results suggest that the prolongation of expiration seen during AB's is primarily due to the increased PIIA. (Supported by NIH HL-25830)

206.8 RESPIRATORY-SPINAL PROJECTIONS TO CAT'S LUMBAR CORD. A.D. Miller, K. Ezure* and I. Suzuki*. The Rockefeller University, New York, N.Y. 10021.

The control of abdominal musculature was investigated by testing the possible projection of medullary and upper cervical respiratory neurons to the lumbar cord. Lumbar-projecting respiratory neurons are likely to affect the activity of abdominal muscles which are innervated in part from L1-3 while the other major respiratory muscles are supplied from more rostral segments. Data were obtained from 31 unanesthetized, decerebrate cats. Neurons (N = 412) were tested for antidromic activation from L1 and sometimes, in addition, L4-5. Neurons were classified as expiratory (E) (N = 171), inspiratory (I) (N = 195), or phase-spanning (N = 46), depending on the relationship of their firing pattern to that simultaneously recorded from the phrenic nerve.

No phase-spanning neurons were found that projected to the lumbar cord.

There was a sparse projection of widely distributed I neurons to the upper lumbar cord. Ten neurons were activated from L1, but not from L4-5. One was located in the dorsal respiratory group (out of 36 DRG cells tested (3%)), 3 were in the ventral respiratory group (VRG) caudal to the obex (out of 29 tested (10%)), and 2 were in C1-2 (out of 7 tested from mid C1 to caudal C2 (29%)); 4 recordings were from axons.

E neurons projecting to the contralateral lumbar cord were distributed in the VRG between the obex and rostral C1 (46 out of 79 cells (58%) were activated from L1). Several of these neurons were activated at low thresholds from lamina VIII and IX in the L1-2 gray matter. One-third of the E neurons that projected to L1 could also be activated from L4-5 (13 out of 40 tested). The concentration of neurons activated from L4-5 was higher in the portion of the VRG nearest to C1 (3.8-7.6 mm caudal to obex). The firing patterns of several neurons were examined in more detail. Thirty-nine E neurons in the VRG caudal to the obex were identified as augmenting; 27 (69%) were activated from L1 while 4 out of 34 (12%) went to L4-5. In contrast, only 9 E cells were identified as decrementing; 2 were activated from L4-5. The strength of possible connections between descending respiratory neurons and abdominal motoneurons remains to be determined.

Supported by grants from NASA (NAG2164, NSG2380) and NIH (NS02619).

206.9 EFFECTS OF ELECTRICAL AND CHEMICAL STIMULATION OF THE RAPHE OBSCURUS ON PHRENIC NERVE ACTIVITY. J.R. Holtman, Jr., N.C. Anastasi* and K.L. Dretchen. Dept. of Pharmacology, Georgetown U Schs. of Med. and Dent., Washington, D.C., 20007.

We have recently shown, employing retrograde tracing techniques, that the raphe nuclei project to the phrenic motor nucleus in the cat (Neurosci. Lett. 44: 105, 1984). These data suggest that the raphe nuclear areas may be involved in the control of respiration. To examine this possibility, we have electrically and chemically (L-glutamate microinjection) stimulated the raphe obscurus (RO) (a heaavily labelled area) while recording phrenic nerve activity in the cat.

Phrenic nerve activity was recorded from a C5 nerve root in chloralose (80 mg/kg) anesthetized, paralyzed and artificially ventilated cats (2-4 kg). Neural discharge was quantitated by using integrated phrenic nerve activity (IPNA). In addition, blood pressure (BP) was monitored from a femoral arterial cannula. An electrode or micropipette were stereotaxically placed in the RO.

Electrical stimulation of the RO was performed for 30 sec over a range of currents (18-144 uA) and frequencies (5-40 Hz) with stimulation pulses of 100 usec duration. Significant ($p<0.05$) increases in both the IPNA amplitude and rate of nerve firing occurred which were dependent upon the current intensity and frequency of stimulation. The maximal increases in IPNA amplitude and rate of firing were 47 ± 17% (N=6) and 146 ± 48% (N=5), respectively. A significant ($p<0.05$) hypotension was also produced and found to be dependent upon the current intensity and frequency of stimulation. The maximal decrease in BP was 51 ± 13 mmHg (N=8). HR did not change. To insure that the changes seen in IPNA and BP were due to stimulation of cell bodies and not fibers of passage, L-glutamate (1M, 2M) was microinjected (100nl) into the RO. Significant ($p<0.05$) dose-related changes in IPNA amplitude occurred with an increase of 44 ± 13% at 1M (N=6) and 80 ± 13% at 2M L-glutamate (N=5). No change in rate of nerve firing occurred. Significant ($p<0.05$) changes in BP also occurred with an increase of 27 ± 6mmHg (N=5) at 1M and 38 ± 14 mmHg (N=6) at 2M L-glutamate. HR did not change.

The data indicate that the RO is involved in respiratory control, influencing phrenic nerve amplitude but not rate of firing. In addition, the RO is involved in cardiovascular control functioning as a vasopressor area. (HL 30849).

206.10 ORBITAL CORTEX STIMULATION ALTERS RESPIRATORY CYCLE TIMING IN THE DRUG-FREE CAT. J.D. Marks and R.M. Harper. Brain Research Institute and Department of Anatomy, UCLA, Los Angeles, CA 90024.

Volitional respiratory rate and effort changes suggest that forebrain areas can affect respiratory rhythmogenesis. The orbital cortex may be one of these areas, since stimulation of that forebrain site in anesthetized preparations produces cessation of respiratory movements. The nature of the respiratory response (i.e., apneic or apneustic) is not known in the unanesthetized preparation. We examined the effect of orbital cortex stimulation on respiratory cycle timing.

Adult female cats had electrodes surgically implanted under halothane-nitrous anesthesia. Teflon-coated stainless-steel stranded wires were placed in costal portions of the diaphragm for recording respiratory EMG. Concentric bipolar stimulating electrodes were stereotaxically implanted bilaterally in the orbital cortex. The raw diaphragmatic EMG was band-pass filtered (30 Hz to 1 kHz), rectified, and integrated by a signal conditioner. Data were recorded onto polygraph paper and were digitized along with the times of occurrence of the stimulus pulses. The stimulus consisted of a 60 Hz train of 40 constant current pulses (300 uamperes, 0.5 msec). Stimuli were delivered at four different points in the respiratory cycle, as determined by the integrated diaphragmatic EMG: early inspiration, late inspiration, early expiration, and late expiration. Mock control trains, delivered at the same points in the respiratory cycle, were also recorded two breaths prior to each stimulation. Sections of the record (4 sec) starting 1.5 sec prior to each stimulation and each control train were extracted for analysis.

Stimulation delivered during late expiration or early inspiration were effective in changing diaphragmatic EMG amplitude and timing. Late expiratory stimulation delayed inspiratory onset for the duration of the stimulus. Early inspiratory stimulation completely inhibited diaphragmatic EMG for the duration of the stimulation, resulting in two inspiratory bursts, i.e., before and after train delivery. Stimuli delivered in late inspiration or early expiration were not effective in changing EMG amplitude or timing.

These results provide evidence that the orbital cortex is a forebrain area that can significantly affect generation of the respiratory rhythm and effort.
Supported by HL 22418-07.

206.11 STIMULATING GRACILIS MUSCLE GROUP III AND IV AFFERENTS REFLEXLY DECREASES TOTAL PULMONARY RESISTANCE IN DOGS. K.J. Rybicki* and M.P. Kaufman* (SPON: D.C. German). Dept. of Physiol., Univ. of Texas Health Science Center at Dallas, Dallas, TX 75235

Stimulation of group III and IV skeletal muscle afferents is well known to reflexly increase ventilation. Little is known, however, about the reflex effect of stimulating these afferents on airway caliber. Although previous investigations have shown that stimulating group III and IV muscle afferents reflexly decreases transverse tension from the trachealis muscle, this variable gives no functional information about airway caliber. We therefore electrically stimulated gracilis muscle afferents in paralyzed, chloralose anesthetized dogs while recording total pulmonary resistance (TPR) which was calculated "on-line" by a Buxco pulmonary analyzer. We tested the hypothesis that stimulating group III and IV afferents decreased TPR. Both gracilis nerves, which supply afferents to hindlimb skeletal muscle, were electrically stimulated at 20 Hz, 0.3 ms duration and at current intensities of 3,5,20,70 and 200 times motor threshold (MT). Compound action potentials were recorded to determine which afferents were stimulated by these current intensities. We found that stimulating group I and II afferents (3xMT) had no effect on TPR. Stimulating group III afferents (5,20,70xMT) significantly decreased TPR. Stimulating group III and IV afferents (200xMT) decreased TPR significantly more than did stimulating group III afferents alone. In addition stimulating these group III and IV afferents (200xMT) at 2 and 5 Hz also significantly decreased TPR.

The decrease in TPR evoked by electrically stimulating group III and IV gracilis muscle afferents was unaffected by propranolol (1.5 mg/kg; iv) but was abolished by atropine methyl nitrate (1.0 mg/kg; iv) which eliminated resting tone to the airways.

We conclude that stimulating group III and IV gracilis muscle afferents, in dogs, reflexly decreases total pulmonary resistance, an effect likely due to the withdrawal of cholinergic tone to the airways. Supported by NIH Grant #HL30710, NIH Program Project Grant #HL06296, and American Heart Association Grant in Aid #83-1179.

206.12 EFFECTS OF BOMBESIN ON RESPIRATORY REGULATION IN THE RAT. J. A. Hedner,* B. T. Hedner,* G. R. Breese, T. J. McCown, and R. A. Mueller (SPON: J. F. Howard, Jr.). Dept. Clinical Pharmacology, Univ. Göteborg, Göteborg, Sweden, and Dept. Anesthesiol., Univ. of North Carolina, Chapel Hill, NC 27514.

Recently, some different peptide neurotransmitters such as substance P, TRH, CCK, and endorphines have been shown to affect respiratory performance when administered into the CNS. These peptides, as well as another putative peptide neurotransmitter, bombesin (BOM), exist in appreciable amounts in the brain stem area. Since this is one of the proposed locations for the respiratory regulating center, we have studied the effects on ventilation of BOM in the rat after intracerebroventricular as well as after local application in the area of the nucleus of the solitary tract (NTS).

Animals were studied in a whole body plethysmographic model under light halothane anesthesia. Cannulae were implanted into the lateral ventricles or into the brain stem at least 48 hours prior to the experiments. Ventilation was estimated by calculation of the following respiratory parameters: respiratory frequency (f), tidal volume (V_T), duration of inspiration (T_I), expiration (T_E) and total respiratory cycle (T_{TOT}). The quotients "inspiratory drive" (V_T/T_I) and "respiratory duty cycle" (T_I/T_{TOT}) were also calculated. Arterial samples were withdrawn for estimation of blood gases.

BOM administered into the lateral ventricle induced a dose dependent immediate (within 3 min.) increase in V_T and a progressive fall in f. Inspiratory drive increased while respiratory duty cycle remained unchanged. As a result of these changes minute ventilation was slightly increased. After administration into the NTS areas (0.5µg/0.5µl) hyperventilation as reflected by a marked respiratory alkalosis developed within 5 min. These effects were due to an increase both in V_T as well as f and were dependent of the specific site of injection in area of the NTS.

These results strongly indicate that BOM acts as a stimulant of respiratory activity by effects within the CNS. At least part of these effects seem to be elicited within the brain stem area.

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206.13 PROTECTION BY PHENYTOIN AGAINST THE TOXIC EFFECTS OF ORGANOPHOSPHATES ON CENTRAL RESPIRATORY CENTERS. K.A. Marx*, N.C. Anastasi*, Y.M. Hernandez*, K.B. Fivozinsky*, A. Raines, K.L. Dretchen. Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007.

We have recently shown that the calcium channel blockers, verapamil and phenytoin (PN), increased the protective effects of atropine (AT) and protopam chloride against organophosphate toxicity in mice (Fed. Proc. 43: 533, 1984). However, these experiments could not distinguish whether the protective effects of the calcium channel blockers were due to action of these compounds at peripheral or central sites. In order to accomplish this we recorded EEG activity and neurally stimulated gastrocnemius muscle contractions in artificially respirated, alpha-chloralose anesthetized cats. Diisopropyl fluorophosphate (DFP) was administered i.v. in incremental doses and the amount of drug necessary for neuromuscular failure (NMF) and maximum seizure activity was determined. DFP alone resulted in NMF at 4.2 mg/kg and EEG activation at 3.6 mg/kg. PN increased maximal seizure activity from 3.6 to 4.8 mg/kg, an increase of 33%. AT increased this activity by 177%. Together, the maximal seizure activity was increased by 350%.

These studies indicated that the CNS is more sensitive than the neuromuscular junction to the toxic effects of DFP and that the calcium channel blockers can protect against this. Since the major cause of death of these compounds is to produce respiratory depression, we investigated the effects of DFP on central respiratory outflow by recording neural activity extracellularly from the phrenic nerve concomitant with gastrocnemius muscle contractions. In all conditions, cessation of phrenic neural discharges occurred prior to blockade of the neuromuscular junction. AT elevated the dose of DFP necessary to block phrenic firing from 2.3 to 6.1 mg/kg, while pretreatment with PN increased the dose of DFP to 3.5 mg/kg. The combination of AT and PN increased the dose of DFP to 8.8 mg/kg.

These data suggest that respiratory depression produced by toxic doses of DFP is due to the action of this agent at central, rather than peripheral, sites. The protection afforded by the calcium channel blockers is probably due to an effect on central respiratory centers.
(ONR N00014-83K-0047)

206.14 PHYSIOLOGICAL PROPERTIES OF DIAPHRAGM MOTOR UNITS. M. Fournier* and G.C. Sieck. City of Hope Medical Center, Duarte, CA 91010 and UCLA, Los Angeles, CA 90024.

Physiological properties of diaphragm motor units were studied in barbiturate anesthetized cats. In an initial study, the spatial distribution of muscle fibers innervated by the right C5 ventral root was determined using the glycogen depletion method. After cervical lamenectomy, medullary and reflex inputs to the phrenic motoneuron pool were eliminated by spinal cord transections at C2 and T1 and by dorsal root section (C3 to C7). Thereafter, cats were mechanically ventilated. The C5 ventral root was stimulated with trains of 100 pps for 100 ms duration repeated at 500 ms intervals. Throughout the period of stimulation (approximately 1 to 2 hours), diaphragmatic EMG responses were detected in the sternal and costal regions but not in the crural. Depletion of glycogen within the stimulated fibers was analyzed histochemically using the periodic acid-Schiff (PAS) reaction. Comparison with the unstimulated contralateral hemidiaphragm, revealed a negatively stained zone originating in the right half of the sternal area and extending to the mid-costal region. This confirmation of the limited territory of innervation by the C5 root permitted optimization of motor unit tension measurements through alignment of the force transducer. Isometric force measurements were made by fixing the central tendon with a clamp and attaching a force transducer in series with the ventral costal margin. The optimal fiber length for maximal force production was set prior to C2 section while the animals breathed spontaneously. Filaments from the C5 ventral root were dissected progressively in a rostro-caudal direction. This systematic approach revealed the presence of a ventral to dorsal distribution of motor units based upon evoked EMG responses. In 5 cats, contraction times (CT) of 31 motor units ranged from 25 to 100 ms (median: 40 ms). Twitch tensions ranged between 0.3 and 8.5 g (median: 0.8 g). Tetanic tensions ranged between 0.8 and 25.0 g (median:3.5 g). The average twitch to tetanic ratio was 0.28.'Sag' was detected in 11 of the 19 motor units where substetanic stimulation was studied. The mean CT of those units showing sag was 35 ms while the mean CT of those not showing sag was 49 ms. Fatigue indices, determined in 13 units, were mostly intermediate. These results, although preliminary, suggest that the contractile properties of motor units in the diaphragm are similar to those of other skeletal muscles. However, there are indications that motor units in the diaphragm may differ in their fatigue resistance.
Supported by NIH Grant HL29999.

207.1 CIRCULATING CATECHOLAMINES AND CARDIOVASCULAR ACTIONS OF INTRATHECAL ARGININE VASOPRESSIN. C.L. Riphagen, L. Bauce* W.L. Veale & Q.J. Pittman. Dept. of Pharmacology and Therapeutics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 141

Anatomical studies have revealed the presence of fibres immunoreactive for arginine vasopressin (AVP) in the intermediolateral cell column region of the spinal cord. We have shown previously that intrathecal administration of AVP into the thoracic region elevates both blood pressure and heart rate in anaesthetized rats. These responses are not blocked by peripheral administration of AVP antagonist which suggests that they may be neurally mediated. To determine whether the cardiovascular responses elicited by intrathecal AVP are due to increased sympathetic tone as revealed by increased levels of circulating catecholamines we measured the plasma levels of norepinephrine (NE) and epinephrine (EPI) in blood samples taken from anaesthetized rats before and after intrathecal AVP administration.

Male Sprague-Dawley rats were anaesthetized with Inactin (0.12g/Kg, i.p.). A cannula (PE-10) for peptide administration was threaded down the spinal subarachnoid space, via an incision in the atlanto-occipital membrane, to the T9 - T11 region. A cannula (PE-60) placed in the carotid artery was used to monitor blood pressure and heart rate and was also used for blood withdrawal. The plasma from each 2 ml blood sample was collected and stored at -70°C pending processing. The red blood cells, made up to 2 ml volume in warm 0.9% NaCl were immediately injected back into the animals. The plasma levels of NE and EPI were measured by HPLC/EC detection.

The animals were allowed to equilibrate for 30 min. after surgery before the first blood sample, used to establish baseline levels of the catecholamines, was obtained. 30 minutes later AVP (5-10 picomoles), in a vehicle of 5-10 µl artificial CSF was administered via the spinal cannula. Two min. later, when blood pressure and heart rate were increasing, the second blood sample was taken. In control animals vehicle only was administered. Following a further 30 min. period, a third blood sample was taken.

Intrathecal administration of AVP (n=5) did not significantly alter the circulating levels of NE or EPI (P>0.1 for both catecholamines)when compared with the levels determined following administration of vehicle only (n=4).

These results suggest that the effects of intrathecal AVP on BP and HR do not involve mechanisms which significantly alter plasma catecholamine levels. Supported by MRC & AHFMR.

207.2 VASOPRESSIN FACILITATES BAROREFLEX INHIBITION OF SYMPATHETIC NERVE ACTIVITY IN RABBITS. P.G. Schmid, G.F. Guo*, and F.M. Abboud*. Veterans Administration Medical Center, Cardiovascular Center and Department of Internal Medicine, University of Iowa, Iowa City, IA 52240.

We determined whether circulating vasopressin (AVP) modulates baroreflex control of lumbar sympathetic nerve activity (LSNA) in anesthetized rabbits. Short term infusion of AVP facilitated reflex inhibition of LSNA compared to phenylephrine (PE). The responses to AVP and PE were abolished by sino-aortic baroreceptor denervation, indicating the facilitation by AVP is baroreflex dependent. We further determined whether AVP can act centrally, peripherally, or both to exert this modulation. We found that except for the low dose of AVP (8 mU/kg/min), infusion of AVP (16 or 32 mU/kg/min) caused greater inhibition of LSNA when compared with PE at given increases in aortic baroreceptor activities, suggesting a central action of AVP. This central action was supported by the observation that, when the carotid sinuses were vascularly isolated, intravenous infusion of AVP augmented carotid baroreflex inhibition of LSNA. On the other hand, AVP may also facilitate baroreflex inhibition of LSNA through sensitization of arterial baroreceptors because intravenous infusion of AVP caused greater excitation of aortic baroreceptors than did PE at given changes in arterial pressure. In some rabbits, intravenous infusion of AVP also elevated the level of aortic baroreceptor activities during increases in arterial pressure induced by intra-aortic balloon occlusion. Furthermore, when AVP was confined to the isolated carotid sinuses, the reflex inhibition of LSNA during distension of carotid sinuses was augmented. Thus, our data suggest that circulating vasopressin may facilitate baroreflex inhibition of sympathetic nerve activity. This facilitation is mediated by sensitization of the afferent limb as well as the central component of the baroreflex. (Supported by the Veterans Administration and NIH HL-14388).

207.3 BARORECEPTOR REFLEX MODULATION BY A5 NORADRENERGIC (NE) NEURONS IN CONSCIOUS RATS. R.L. Stornetta*, R.M. McCarty* and P.G. Guyenet. Neuroscience Program, Univ. of Virginia, Charlottesville, VA 22906.

The pontine A5 NE cells have been suggested to participate in cardiovascular control, yet their exact role remains controversial. Part of the confusion may be due to the use of anesthetized animals. In the present study, freely behaving rats were used to evaluate the effects of A5 lesions on baroreceptor reflexes (BRFX).

Male Sprague-Dawley rats were anesthetized with pentobarbital and microinjections of 6OHDA-HBr(1µl,6 µg/µl) or vehicle (ascorbic acid/saline) were placed bilaterally into the A5 cell group. Ten days to 2 weeks later, the animals were reanesthetized with pentobarbital and the tail artery and jugular vein were cannulated with pe tubing which was externalized to record BP and HR and inject drugs without disturbing the animals. Twenty-four hours later, the conscious animals were tested in individual cages. Blood pressure and heart rate were recorded continuously. Five doses each of nitroprusside (NP) and phenylephrine (PE) were administered i.v. and the resulting HR and BP were recorded. BP and HR were allowed to return to baseline values between doses. Baseline values for BP (lesion x=114 mm Hg±2.1(SEM), n=18;control x=114±2.3, n=19) and HR x=357 beats/min ±6.2; control x=347±5.6 were not different between groups. BP changes induced both by PE and NP were also the same for both groups. All lesions were verified after the experiment by counting catecholamine fluorescent cells in A5. Multiple analysis of variance with repeated measures revealed a significant interaction (F=5.77,p=.02,df=1) for the change in HR elicited by the drugs between the 2 groups (lesion and control). The groups did not differ in HR response to PE but a significant interaction was found for groups by doses of NP (F=2.29,p=.03,df=4.32). The HR response of lesioned animals was less than that of controls for all doses (significantly different for the highest dose of NP, t=3.25,p=.003,df=35).

This result indicates that lesioned rats exhibit a decrease in the reflex tachycardia elicited by reductions in BP, a predominantly sympathetic response. Since the PE-induced bradycardia was not affected by the lesion, this predominantly parasympathetic component of the BRFX remained intact.

In conclusion, these results suggest that A5 NE cells increase the gain of the sympathetic component of the BRFX. (HL 28785)

207.4 COMPARISON OF THE CENTRAL CARDIOVASCULAR EFFECTS OF ANGIOTENSIN AND BRADYKININ: ROLE OF CHOLINERGIC NEURONS. M. Serra* and J.J. Buccafusco. Depts. of Pharmacology and Toxicology, and Psychiatry, Medical College of Georgia and V.A. Medical Center, Augusta, GA 30912.

Both angiotensin (ANG) and bradykinin (BK) are pressor agents when introduced directly into the CNS. The purpose of this study was to compare the cardiovascular changes in response to intracerebroventricular (icv) injection of each peptide into conscious, freely-moving rats; and, since central cholinergic stimulation often results in a hypertensive response in conscious rats, to examine the role of central cholinergic neurons in mediating these actions. Outbred female rats were surgically prepared first with chronic icv cannula guides and 1 week later with indwelling arterial catheters. Icv injection of 5 µg of BK produced an immediate peak increase in blood pressure (BP) of 62±6/49±4 mmHg and a concomitant increase in heart rate (HR) of 79±11 beats/min. BP and HR were elevated for 10-15 min. In contrast, icv injection of 10 µg of ANG produced a smaller increase in BP (29±2/30±4mmHg) but the response was of longer duration (up to 50 min). Also, the pressor response was accompanied by a decrease in HR of 75±19 beats/min.

To determine the role of central cholinergic neurons in mediating these responses hemicholinium-3 (HC-3) was used to deplete endogenous acetylcholine levels. Icv injection of 20 µg of HC-3 one hr before BK almost completely abolished the pressor response to the peptide (7±2/4±2mmHg) and converted the increase in HR to a decrease (-47±10 beats/min). Similar pretreatment with HC-3 also inhibited the pressor response to ANG, however the effect was not as marked (14±4/16±3mmHg; ~50% inhibition). The fall in HR was also reduced (33±12 beats/min). HC-3 itself had no effect on BP but produced a significant fall in HR.

These results indicate that ANG and BK elicit different patterns of cardiovascular changes following central injection in conscious rats. The actions of both peptides appear to require intact functioning cholinergic neurons, however, at least a portion of the response to ANG may be mediated via other neuronal systems. Supported by HL 30046.

207.5 ENHANCED DEPRESSOR EFFECT OF MUSCIMOL IN THE DOCA/NaCl HYPERTENSIVE RAT. S. Nagahama* and S. Oparil* (SPON: Dr. Sherry Winternitz). Cardiovascular Research and Training Center, University of Alabama, Birmingham, AL 35294.

Intracerebroventricular (ICV) administration of GABA has been reported to decrease blood pressure (BP) by reducing sympathetic vasomotor tone through a central mechanism in rat and cat. To examine the participation of GABAergic mechanisms in deoxycorticosterone acetate (DOCA)/NaCl hypertension, the effects of muscimol (50-1000 ng, ICV), a GABA agonist, on mean arterial pressure (MAP), and plasma norepinephrine (NE) and epinephrine (E) were examined in DOCA/NaCl hypertensive rats. The depressor effect of hexamethonium (30 mg/kg, IA) was used to assess sympathetic control of BP. Uninephrectomized male Sprague-Dawley rats (6 wks old) were implanted with DOCA (SC, 100 mg/kg) and given 1% saline to drink for 4 wks. Age matched uninephrectomized rats given tap water to drink were normotensive controls (H_2O controls). Spontaneously hypertensive rats (SHR) of the Okamoto strain (10 wks old) were hypertensive controls. Experiments were performed in conscious, unrestrained animals 48 hrs after placement of cannulas into femoral artery and lateral ventricle.

MAP in DOCA/NaCl rats was significantly greater than in H_2O controls (168 ± 3 vs 121 ± 2 mmHg, $P < 0.01$) and almost the same as in SHR (163 ± 3 mmHg). Muscimol caused dose-dependent decreases in MAP in all groups. The depressor action of muscimol in the DOCA/NaCl rat was significantly greater than in H_2O controls and SHR.

	NE (pg/ml)	E(pg/ml)	maximum Δ MAP(%) to hexamethonium
DOCA/NaCl	(A) 409±45**	223±39*	53±2**
	(B) 464±44*	75±18##	
H_2O	(A) 222±26	114±25	45±2
Control	(B) 303±30	53±15#	

(A) before muscimol injection, (B) 15 min after muscimol (1000 ng IVC) injection.
*$p < 0.05$, **$p < 0.01$ as compared with H_2O controls.
#$p < 0.05$,##$p < 0.01$ as compared with (A)

The increased basal levels of NE and E and enhanced depressor responses to hexamethonium and muscimol in DOCA/NaCl hypertensive rats suggest that DOCA/NaCl rats have increased sympathetic tone which may be related to altered GABAergic activity.

207.6 HIGH CONCENTRATION OF NEUROPEPTIDE Y IMMUNOREACTIVE FIBERS IN THE MEDIAN PREOPTIC NUCLEUS. D.K. Hartle and J.K. McDonald, Depts. of Physiology and Anatomy, Emory Univ. Sch. of Med., Atlanta, GA 30322.

The median preoptic nucleus (MePON) appears to be an important integration center for humoral and neural cardiovascular information and has been implicated in the mechanism of several types of experimentally induced hypertension (Hartle & Brody, Circ. Res., 1984). The MePON receives ascending catecholaminergic innervation from the ventrolateral medulla , locus coeruleus and nucleus of the solitary tract, as well as other input from several hypothalamic nuclei (paraventricular, arcuate, preoptic, anterior and periventricular) (Saper & Levisohn, 1983). Since avian and bovine pancreatic polypeptide (APP & BPP) and recently neuropeptide Y (NPY)-like immunoreactivities have been localized in several of these sources of afferent input we have examined the MePON for the presence of NPY immunoreactivity.

Male albino rats (300g, Holtzman) were perfused through the aorta with fixative (4% paraformaldehyde, 0.01 M Na periodate, 0.1 M lysine HCl) in 0.1M phosphate buffer at pH 7.4, 23° C. The brains were removed, fixed for 4-6 hr and then placed in 30% sucrose buffered phosphate overnight at 4°C. Horizontal sections (20-40 μ) were cut through the MePON and processed for immunohistochemistry. NPY antiserum was generously provided by P. Emson (Cambridge, U.K.). Preabsorption of the antiserum with 10^{-6} M synthetic porcine NPY (Peninsula) prevented labeling. The MePON contained a dense plexus of NPY immunoreactive fibers throughout its dorso-ventral extent. These fibers appeared to be continuous with fibers located in a compact periventricular zone extending from the region of the paraventricular nucleus posteriorly, to the MePON, anteriorly. In addition, a diffuse band of labeled fibers entered the MePON from a postero-lateral direction through the medial preoptic nuclei. Sections through the dorsal MePON revealed a network of varicosed NPY fibers rostral to the anterior commissure. The origin of the positive fibers observed in this study is unknown. NPY reportedly exerts modulatory effects on catecholaminergic neurotransmission. In view of its colocalization with catecholamines in important brainstem cardiovascular regions which innervate the MePON, the presence of NPY labeled fibers in the MePON suggests a role for NPY in the integrated functions of this nucleus. (Supported by Emory Univ., PHS 5F32HL0668502 and GA Heart Assoc).

207.7 ALTERATIONS OF BLOOD PRESSURE AND WATER BALANCE FOLLOWING DESTRUCTION OF CATECHOLAMINE INPUT TO SUPRAOPTIC NUCLEUS: DIFFERENTIAL EFFECTS OF NOREPINEPHRINE VERSUS DOPAMINE. B.J. Davis., C.D. Sladek and J.R. Sladek, Jr., Depts. of Anatomy and Neurology, University of Rochester, Rochester, NY 14642.

We reported that destruction of catecholamine (CA)-containing pathways to the supraoptic nucleus (SON) via bilateral injections of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB) and supraoptic decussations (SOD) led to reduced blood pressure (BP), marked reductions of water intake, and a failure to conserve fluids when given gastric intubations (decreased fluid intake/urine output ratio - I/O).* Since both norepinephrine (NE)- and dopamine (DA)-containing fibers are located in the region of the SON, it was of interest to determine which of these CA fiber systems contributed to the functional deficits observed following lesioning. To address this question, animals were given 6-OHDA injections into the MFB and SOD following pretreatment with the NE uptake blocker, desmethylimipramine (DMI), which protects the NE fibers. Four groups of adult, male rats were studied. Lesioned (L) rats (n=9) received bilateral 6-OHDA (6 ug/site) in the MFB and SOD. Sham lesioned (S) rats (n=8) received bilateral cannula placements in the MFB and SOD. Lesioned-DMI (LD) rats (n=7) received bilateral 6-OHDA (6 ug/site) in the MFB and SOD 20 minutes following DMI (20 mg/kg, i.p). Sham-DMI (SD, n=8, received bilateral cannula placements in the MFB and SOD 20 minutes following DMI (20 mg/kg, i.p.). BP was monitored by tail cuff prior to and 3 days following treatment. The percent change of BP was used to evaluate the effects of treatments on BP. I/O was determined 3 days following treatment. Compared with S rats, L rats showed a marked reduction of water intake and a failure to conserve fluids when given gastric intubations (decreased I/O). LD rats also differed significantly from S rats in these parameters but were not different from L rats in terms of water intake or I/O. Since DMI by itself did not appear to alter water balance in the SD group, these results suggest that the 6-OHDA induced alterations in fluid balance were secondary to the destruction of DA rather than NE fibers. The opposite was true for the effects of 6-OHDA on BP. As in our previous study*, the BP of L rats was significantly lower than that of S rats. However, BP of LD rats was comparable to that of S and SD rats. Since DMI pretreatment blocked the effect of 6-OHDA lesions on BP, reduction of BP following denervation of CA input to SON probably reflects destruction of NE fibers.

*Davis, et al., Anat. Rec. 208:42A.

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207.8 BOMBESIN ALTERS THE CARDIOVASCULAR RESPONSE TO COLD EXPOSURE. L.A. Fisher and M.R. Brown. Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

The tetradecapeptide, bombesin (BOM), has potent central nervous system (CNS) actions on thermoregulation in mammals. The CNS effects of bombesin on body temperature (T_b) vary in parallel with ambient thermal conditions, i.e., hypothermia during cold exposure, hyperthermia during heat exposure, and no change at thermoneutrality. In cold-exposed animals, BOM produces hypothermia by preventing normal increases in oxygen consumption (VO_2) and thus inhibiting regulatory heat production. Since regulatory heat production may depend in part on cardiovascular adjustments, we examined the CNS effects of BOM on mean arterial pressure (MAP), heart rate (HR), T_b and VO_2 during cold exposure.

All experiments were performed in conscious, unrestrained male rats fitted with lateral cerebroventricular cannulae and jugular venous and femoral arterial catheters. MAP and HR were monitored for 60 min at room temperature (22°C). Following measurement of T_b and intracerebroventricular (icv) injection of test compounds, the rats were transferred to a metabolic chamber maintained at 7.5°C and MAP, HR and VO_2 were recorded for 60 min, whereupon T_b was measured. Rats receiving icv injections of vehicle displayed significant increases in MAP, HR and VO_2 and no change in T_b during cold exposure. BOM-treated (5.7 pmoles - 5.7 nmoles) rats exhibited appropriate increases in MAP but had dose-related reductions of HR, VO_2 and T_b compared to controls. HR and VO_2 were positively correlated ($r = 0.93$) in bombesin-treated rats. The CNS-selective somatostatin analog, ODT8-SS, which is demonstrated to antagonize the effects of BOM on VO_2 and T_b also restored cold-induced tachycardia in bombesin-treated rats. HR and VO_2 were positively correlated ($r = 0.87$) in rats receiving vehicle, BOM, ODT8-SS, and BOM plus ODT8-SS. Atropine methyl nitrate treatment (1 mg/kg, iv) failed to antagonize the effects of BOM on HR, VO_2 and T_b during cold exposure.

The results demonstrate that icv administration of BOM inhibits cold-induced elevations of HR and VO_2. If cold-induced elevations of VO_2 are dependent on tachycardia and increased cardiac output, BOM may inhibit regulatory heat production and produce hypothermia in part by suppressing the cardiac response to cold exposure.

207.9 (+)-PHNO, CARDIOVASCULAR ACTIONS OF A NEW DOPAMINE AGONIST IN SPONTANEOUSLY HYPERTENSIVE RATS. G. E. Martin and E. V. Lis.* Merck Sharp and Dohme Research Laboratories, West Point, PA 19486

The effects of the potent and selective dopamine agonist, (+)-n-propyl-9-hydroxy-naphthoxazine (+)-PHNO on cardiovascular parameters in the unanesthetized spontaneously hypertensive rat (SHR) were ascertained. (+)-PHNO was given either p.o. or i.p. to Wistar-Okamoto SHRs in which chronic indwelling tail artery catheters had been implanted at least one day earlier. Mean arterial pressure (MAP) and heart rate were continuously monitored in free-moving rats using a Buxco Cardiovascular analyzer.

Given p.o., (+)-PHNO caused a significant fall in MAP ($\bar{X}$ fall = 30-40 mm Hg) in a dose range of 12.5 to 200 μg/kg. The fall in MAP persisted for 1 to 2 hr. No marked change in heart rate was produced. Given i.p., (+)-PHNO also produced falls in MAP of a similar magnitude. The minimum effective dose for a significant fall in MAP to occur was 6.25 μg/kg. A short-lived bradycardia was observed following some doses of (+)-PHNO given i.p.

Pretreatment with the dopamine receptor antagonists haldol (0.1 mg/kg, i.p., -30 min) or l-sulpiride (9 mg/kg, i.p., -30 min) significantly reduced the fall in MAP produced by (+)-PHNO (50 μg/kg, i.p.) as well as the bradycardia elicited by the compound. The peripherally selective dopamine receptor blocking agent domperidone (10 mg/kg, i.p., -30 min) shifted the dose response curve for (+)-PHNO-induced falls in MAP to the right. L-646,462, another peripherally selective dopamine receptor antagonist, produced a dose-related inhibition of (+)-PHNO-induced (50 μg/kg, i.p.) falls in MAP over the dose range of 1.25 to 5 mg/kg, i.p. (-30 min). The selective α_2-receptor antagonist, idazoxan (1 mg/kg, i.p., -30 min) did not attenuate the effect of (+)-PHNO (50 μg/kg, i.p.) on the MAP of the SHR.

The results indicate that (+)-PHNO is hypotensive in the SHR and it also seems to produce a shorter lived bradycardia. The naphthoxazine produces these effects via dopamine receptors located both in the central and peripheral nervous system.

207.10 CARDIOVASCULAR ACTIONS OF 8-OH DPAT, A SEROTONIN RECEPTOR AGONIST. N. N. Share,* R. M. Evans,* E. V. Lis* and G. E. Martin (SPON: V.J. Lotti). Merck Sharp and Dohme Research Laboratories, West Point, PA 19486.

The cardiovascular effects of 8-OH DPAT, a compound reported to possess potent CNS serotonin agonist activity (Hjorth et al., J. Neural. Trans., 1982, 55: 169), were examined in chlorolose anesthetized cats and in unanesthetized Wistar- Okamoto spontaneously hypertensive rats (SHR). Given i.v. in cats, both 8-OH DPAT and clonidine, an α_2-adrenergic agonist, evoked similar reductions in blood pressure, heart rate and sympathetic renal nerve activity (RNA). Whereas clonidine's vasomotor depressant activity was clearly reversed by the α_2-receptor antagonists yohimbine and idazoxan, 8-OH DPAT's effects were largely unaltered by these agents. In contrast, the serotonin receptor antagonist methiothepin, completely reversed 8-OH DPATs reduction in RNA without altering clonidine's effect. Methiothepin, which is also hypotensive, failed to alter 8-OH DPAT-induced falls in blood pressure in the cat. Cyproheptadine, a relatively selective antagonist for the S_2 serotonin receptor failed to alter the effects of 8-OH DPAT in the cat.

In SHRs, 8-OH DPAT produced falls in both mean arterial pressure (MAP) and heart rate whether given orally (minimum hypotensive dose = 7.5 mg/kg) or i.p. (0.02). Upon intracerebroventricular (ICV) administration, 8-OH DPAT produced a fall in MAP but no fall in heart rate. Whereas cyproheptadine (5 mg/kg, i.p., -30 min) and methergoline (1 mg/kg, i.p., -30 min) failed to alter the cardiovascular effects of 8-OH DPAT, methiothepin (0.5 mg/kg, i.p., -30 min) blocked the bradycardia produced by 8-OH DPAT (2.5 mg/kg i.p.) but not the hypotension. The latter result is confounded somewhat by the fact that methiothepin by itself produces a fall in MAP. Pretreatment with the peripherally selective dopamine receptor antagonists domperidone (2.5, 10 mg/kg i.p.) or L-646,462 (10 mg/kg i.p.) failed to alter the hypotensive and bradycardic actions of 8-OH DPAT.

The results indicate 8-OH DPAT is a potent hypotensive agent. 8-OH DPAT may exert its cardiovascular effects via a specific subset of serotonin receptors since neither cyproheptadine nor methergoline reversed its effects, whereas methiothepin was active in blocking the decrease in RNA and bradycardia. Clearly its hypotensive effect is not mediated via α_2-adrenergic nor peripheral dopaminergic receptors. Further characterization of serotonin receptor(s) may be required before 8-OH DPAT's site of action is precisely delineated.

207.11 SUBSTANCE P MECHANISMS OF THE SPINAL CORD RELATED TO VASOMOTOR TONE IN THE SPONTANEOUSLY HYPERTENSIVE RAT. Y. Takano, W.B. Sawyer, and A.D. Loewy. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Substance P (SP) mechanisms involved in regulation of vasomotor tone at the spinal cord level in normotensive (WKY) and spontaneously hypertensive rats (SHR) were studied. Our results indicate:

1) Intrathecal injections of the SP antagonist D-Pro^4,D-Trp^{7,9} SP (4-11) cause dose-dependent decreases in mean arterial pressure and heart rate in Sprague-Dawley, WKY and SHRs; the maximal blood pressure decreases are equal to those seen after cervical spinal cord transection;

2) Intrathecal injections of this antagonist into the L1 spinal level in WKYs or SHRs that had previously had their C8 spinal cords transected caused a rise in blood pressure and heart rate, suggesting that intrinsic spinal SP mechanisms are not involved in vasomotor tone;

3) The intermediolateral cell column region (IML) of 16-week-old WKY and SHRs have a single high affinity and saturable binding component with approximately the same dissociation constant (K_d=1.21 nM for WKYs; K_d=1.25 nM for SHRs); the SHRs showed a higher number of sites (B_{max}=24.5 fmole per mg protein) than WKY rats (B_{max}=19.9 fmole per mg protein). The K_d and B_{max} obtained from IML sections from 4-week-old WKY and SHRs exhibit no differences, although their values are higher than those obtained from the 16-week-old animals;

4) D-Pro^4,D-Trp^{7,9} SP (4-11) has the same relatively low (micromolar range) potency for displacing ^{3}H-SP binding in the IML of WKY and SHRs; and

5) SHRs (16 week old) contain 20% more SP immunoreactivity in the IML than WKY rats (834±36 pg/mg protein vs. 694±50 pg/mg protein); 4-week-old rats do not show such differences.

These results indicate the possibility that abnormal substance P mechanisms may be related to the pathogenesis of hypertension in the SHR.

207.12 PHARMACOLOGIC EVIDENCE THAT SUBSTANCE P IS INVOLVED IN BULBOSPINAL CARDIOVASCULAR CONTROL IN THE RAT. J.R. Keeler and C.J. Helke. Dept. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Studies of the neural components of central cardiovascular control show the importance of a projection from the ventral medulla to the intermediolateral cell column (IML) of the spinal cord. Substance P (SP) and serotonin (5-HT) are associated with this pathway, and excite sympathetic preganglionic neurons when iontophoresed into the IML. In addition, SP receptors are present in the IML [Charlton and Helke, this meeting]. Our previous studies showed a sympathoexcitatory pathway which is inhibited by GABA and activated by the GABA antagonist, bicuculline methiodide (BMI), when applied topically to the intermediate area on the ventral surface of the medulla oblongata (VSMO) of the rat. In the present study, intrathecal injections were given acutely (SP antagonists), or 10-14 days prior to the experiment (5,7-dihydroxytryptamine; 5,7-DHT). The effects of these drugs on mean arterial pressure (MAP) and heart rate (HR), as well as their ability to block the responses to topical application of GABA or BMI at the VSMO were assessed in anesthetized, artifically ventilated rats.

These experiments showed SP, but not 5-HT, to be a functional component in the pathway mediating GABAergic responses from the VSMO to the IML. D-Pro^2, D-Trp^{7,9}-SP, D-Pro^2, D-Phe^7, D-Trp^9-SP, and D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}-SP (50 μg) decreased MAP to 2/3 baseline values, but did not change HR. These 3 SP antagonists also blocked the characteristic increases in MAP and HR elicited by BMI. Because of the long-lasting effects of the 50 μg dose of antagonists, a lower dose was used to test their reversibility. D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}-SP (5 μg) decreased MAP to 68% of control, and also inhibited the BMI-induced increases in MAP and HR to 22% and 48%, respectively. The inhibition lasted 1-2 hours. Not all of the putative SP antagonists had the same actions in the spinal cord. A 50 μg dose of D-Pro^4, D-Trp^{7,9,10}-SP(4-11) (about 7 times less potent in binding studies) increased HR and did not change MAP or the BMI-induced cardiovascular responses. Intrathecal injections of the 5-HT neurotoxin, 5,7-DHT (200 μg X 2), resulted in 56% depletion of 5-HT in the thoracic spinal cord, but did not change either basal MAP and HR, nor the responses to BMI and GABA applied to the VSMO. This pharmacologic evidence supports the neuroanatomical, neurochemical and electrophysiologic evidence for a role of spinal cord SP in cardiovascular regulation.

207.13 EFFECTS OF DIETARY Na^+ ON BRAINSTEM ADRENERGIC RECEPTOR BINDING AND ITS REGULATION BY Na^+ IN VITRO IN DAHL SALT-SENSTITIVE AND RESISTENT RATS. P. Ernsberger, D.C. U'Prichard, and S. Azar*. Neuroscience Program and Dept. of Pharmacology, Northwestern Univ., Chicago, IL 60611.

Excess dietary Na^+ in Sprague-Dawley rats alters kidney and brain adrenergic receptor binding and its regulation by Na^+ in vitro (Soc Neurosci Abs 9:1121, 1983). We examined these effects in the Dahl salt-sensitive (DS) rat, which develops hypertension when fed a high-Na^+ diet, and the Dahl resistent (DR) rat which lacks a pressor response to Na^+. 3 DS and 3 DR rats were sacrificed while on low-Na^+ chow, and after 1 or 3 weeks of an 8% NaCl diet. The brainstem including hypothalamus was homogenized in 50 mM Tris-HCl buffer containing 5 mM EDTA and washed twice by centrifugation. Binding assays for alpha$_1$-, alpha$_2$- and beta-adrenergic receptors were conducted as previously described using ^{3}H-prazosin (^{3}H-PRAZ, 1.8 nM), ^{3}H-p-aminoclonidine (^{3}H-PAC, 0.5 nM), and ^{3}H-dihydroalprenolol (^{3}H-DHA, 0.62 nM), respectively, and incubated 20-40 min at 25° with phentolamine (^{3}H-PAC: 1 uM, ^{3}H-PRAZ: 10 uM) or 200 nM (-)-propranolol (^{3}H-DHA) used to define non-specific binding. Data were analyzed by 2-way ANOVA by strain and diet exposure.

DS rats displayed increases in ^{3}H-PAC specific binding (all DS: 83 ± 5 fmol/mg protein, all DR: 59 ± 3) and the proportion of ^{3}H-PAC binding displaced by 25 mM Na^+ (all DS: 67 ± 3%, all DR: 54 ± 5). The increase in ^{3}H-PAC binding in DS rats could be accounted for entirely by a 66% increase in the Na^+-displacable (high-affinity alpha$_2$) fraction, since Na^+-insensitive (low-affinity alpha$_2$) binding was unchanged (all DS: 27 ± 2 fmol/mg protein, all DR: 25 ± 2). Alpha$_1$-receptor binding was also increased (all DS: 89 ± 8 fmol/mg, all DR: 57 ± 6), consistent with previous findings in SHR hypothalamus. Beta-receptors were decreased in DS rats but increased with exposure to the high-Na^+ diet, with strain differences eliminated by 3 weeks (fmol ^{3}H-DHA specific binding/mg protein):

	Low-Na^+	1 wk High-Na^+	3 wks High-Na^+
Sensitive	11 ± 5	19 ± 3	36 ± 8
Resistent	33 ± 2	32 ± 3	43 ± 4

These receptor changes may be related to Na^+-induced hypertension, which is in part neurally mediated (Clin Sci 61:49s, 1981), and Na^+-induced behavioral changes as well as behavioral differences between DR and DS (Behav Neural Biol 37:10, 1983). Supported by a grant from Nova Pharmaceutical.

207.14 ENHANCEMENT OF BULBOSPINAL EXCITATORY TRANSMISSION TO SYMPATHETIC PREGANGLIONIC NEURONS (SPGNs) BY DESIPRAMINE AND BY DEXTROAMPHETAMINE. Chaichan Sangdee*, Scott C. Steffensen*, and Donald N. Franz, Department of Pharmacology, University of Utah School of Medicine, Salt Lake City, Utah 84132.

The present study examined the effects of two drugs that selectively enhance central norepinephrine (NE) transmission, desipramine and dextroamphetamine, on descending excitatory transmission to SPGNs in an effort to determine whether bulbospinal NE pathways are excitatory or inhibitory. Sympathetic discharges recorded from upper thoracic preganglionic rami were evoked at 0.1 Hz by stimulation of descending excitatory pathways in the cervical dorsolateral funiculus of unanesthetized, spinal cats. Drugs were administered intravenously.

Desipramine (1-2 mg/kg) produced gradual increases in transmission to 145-200% of control values which were sustained for several hours. Larger doses (3-5 mg/kg) produced rapid increases in transmission which began to subside within 20 min. Likewise, 0.5 mg/kg of dextroamphetamine produced a gradual increase in transmission to over 300% of control which was sustained for several hours, but 1-2 mg/kg produced comparable, rapid increases which often subsided toward control levels. Enhancement by either drug was sustained for more than 3 hr if alpha-2 receptors were blocked by yohimbine (0.5-1 mg/kg). Increased transmission by either drug was prevented or reversed by chlorpromazine (1 mg/kg).

These results indicate that modest facilitation of NE transmission by blocking NE reuptake with desipramine or increasing NE release with amphetamine produces marked enhancement of descending excitatory transmission to SPGNs, thereby supporting an excitatory function for the NE pathways. However, excessive facilitation of NE transmission by these drugs appears to permit sufficient spill-over of NE into the neuropil to reach remote inhibitory alpha-2 receptors and to produce depression of SPGN excitability as does clonidine. SPGNs may be regulated by two types of adrenergic receptors that are segregated.

(Supported by NIH grants HL-24085 and GM-07579)

207.15 YOHIMBINE-INDUCED ALTERATIONS IN CENTRAL AND PERIPHERAL MONOAMINES IN HYPERTENSIVE (SHR) AND NORMOTENSIVE (WKY) RATS. R. Dawson, Jr., S. Nagahama and S. Oparil. Cardiovas. Research and Training Ctr., Univ. of Alabama in Birmingham, Birmingham, AL 35294.

Yohimbine is a relatively specific α_2 receptor antagonist. Blockade of presynaptic α_2 receptors results in an inhibition of the autoregulatory control of norepinephrine (NE) release. The present studies examined the pharmacological actions of yohimbine in normotensive (WKY) and hypertensive (SHR) rats by evaluating the blood pressure (BP) responses and measuring alterations in monoamine stores after peripheral administration of yohimbine.

Eight week old male SHR (BP=153±10 mmHg) and WKY (BP=126±3 mmHg) rats received yohimbine (10mg/kg,ip) and BP and monoamine levels were determined in separate groups of rats. Yohimbine resulted in a significant drop in BP ($p<0.01$) and significant ($p<0.05$) elevations in plasma NE and epinephrine, while adrenal catecholamine levels were unchanged. One hour after yohimbine, NE levels in spleen were reduced approximately 20% in both SHR and WKY. However, significant ($p<0.01$) reductions in kidney NE levels occurred only in the SHR. SHR had significantly ($p<0.05$) higher basal levels of NE in kidney, cerebellum, and medulla; after yohimbine administration these differences were abolished. Cerebellar NE levels were decreased 42-56% whereas in the medulla NE levels were decreased only 16-27%. In all brain regions and the kidney, the fall in NE levels after yohimbine was greater in the SHR. Serotonin levels were increased and 5-hydroxyindoleacetic acid levels were decreased in the medulla and cerebellum, suggesting a decrease in serotonin turnover after yohimbine administration.

These results suggest a greater presynaptic control of NE release in the SHR compared to the WKY. The finding of regional differences in the effectiveness of yohimbine in reducing NE stores, may be related to regional differences in the density or subtype of α_2 receptors.

This research was supported by NIH grants HL22544 and HL25451.

207.16 ALPHA-2 BINDING SITES IN THE THORACIC SPINAL CORD: AUTORADIOGRAPHIC EVIDENCE FOR POSTSYNAPTIC LOCALIZATION IN THE INTERMEDIOLATERAL CELL COLUMN. J.R. Unnerstall, M.J. Kuhar, R. Grzanna and L.P. Schramm. Dept. Neuroscience, Johns Hopkins Univ. Sch/Med, Balto., MD 21205

Although adrenergic agonists can inhibit the tonic discharge of sympathetic preganglionic neurons (PGNs) by an action at alpha-2 receptors, stimulation of the ventrolateral medulla in the area of the epinephrine (EPI) neurons which innervate the PGNs causes an increase in PGN firing. Because of this anomaly, it is unclear where the alpha-2 receptors are localized in relation to the descending EPI neurons. We used autoradiographic techniques to localize alpha-2 binding sites in relation to PGNs and to obtain evidence for a pre- or postsynaptic localization of these receptors in the intermediolateral cell column (iml).

Female Sprague-Dawley rats were anesthetized, and the spinal cords were surgically transected (T6). Rats were allowed to survive for 1, 5 or 14 days. Alpha-2 binding sites in 10 um horizontal sections were labeled with either 0.6 nM or 20 nM [^{3}H]para-aminoclonidine ([^{3}H]PAC) to identify either the high- or high- and low-affinity sites respectively according to published procedures (Unnerstall et al., Brain Res. Rev. 7:69, 1984).

The highest grain densities in the sections labeled with [^{3}H]PAC were found in the iml between clusters of putative PGNs. Lower grain densities were seen over the neuropil extending from the clusters toward the midline. Significantly higher binding was seen in the iml in upper, as opposed to lower, thoracic segments. Five days following the surgery, a small decrease in binding (10-15%) was seen below the transection when compared to sham-treated rats. After 14 days, a statistically significant increase (73%) was seen in the binding of [^{3}H]PAC below the lesion in sections labeled with both the low and the high concentrations of this ligand. Norepinephrine (NE) and dopamine-beta-hydroxylase activity were reduced to near background levels below the transection by 14 days.

The increase in [^{3}H]PAC binding seen in the iml below the lesion after 14 days may indicate denervation supersensitivity and suggests that most of these alpha-2 binding sites are postsynaptic to NE or EPI neurons. However, the small decrease seen after 5 days suggests that a subpopulation of these binding sites may be on the terminals of bulbospinal axons. (Supported by grants MH25951, DA00266, MH00053, HL16315, NS16654 and the McKnight Foundation).

207.17 ROLE OF α_1 AND α_2 ADRENOCEPTORS OF THE MEDIAN HYPOTHALAMUS ON THE SYSTEMIC ARTERIAL PRESSURE OF RATS. W.A. Saad, S. Sedenho*, J.V. Menani*, William A. Saad*, L.A.A. Camargo* and A. Renzi*. Departamento de Fisiologia e Patologia, F.O.A.-UNESP, Araraquara, SP. e Dept9 de Cirurgia, FMSP-USP, São Paulo.

The median hypothalamus has noradrenaline levels similar to those of other central areas involved in cardiovascular control. Evidence obtained by receptor-labelling techniques have indicated that both α_1 and α_2 receptors occur in brain tissue (Langer, 1980). In the present experiment we compared the effects of yohimbine and prazosin, which selectively antagonize α_2 and α_1 receptors, respectively, on the hypotensive effect of noradrenaline, which is a nonselective agonist (Starke et al., 1975), when injected into the median hypothalamus of rats. A dose-response curve was determined using noradrenaline doses of 10, 20, 40, 80 and 160 nM, which induced increases of 21 to 65 mmHg in arterial pressure in relation to the controls. Yohimbine previously injected at doses of 10 and 40 nM shifted to the right the dose-response curve for adrenaline. The slopes for a l curves were significantly different from zero ($P < 0.05$). PD_{50} values for noradrenaline and yohimbine at doses of 10 and 40 nM were 40.192 and 80 nM, respectively, although mean values for the groups injected with the two yohimbine doses did not differ amongst themselves. Prazosin, also at the doses of 10 and 40 nM, antagonized the hypertensive effects of noradrenaline more intensely than yohimbine. The respective PD_{50}'s for the two prazosin doses were 768 and 10240 nM. These results may be interpreted on the basis of the possible existence of two types of α-adrenoceptors at the presynaptic and postsynaptic sites which may have different affinities for the agonist and antagonist used here. The decreased slope for the dose-response curve for noradrenaline after treatment with prazosin and the high respective PD_{50}'s show greater interaction of the agonist with α_1 adrenoceptors. In view of the fact that α_1-type adrenoceptors have high affinity for prazosin, we may conclude that α_1 adrenoceptors of the median hypothalamus play a more important role than α_2 adrenoceptors in the control of systemic arterial pressure.

207.18 CARDIAC DISTRIBUTION OF α_1-ADRENERGIC AND MUSCARINIC RECEPTOR SITES IN DAHL HYPERTENSION-SENSITIVE (S/JR) AND HYPERTENSION-RESISTANT (R/JR) RATS FOLLOWING PRENATAL OR POSTNATAL EXPOSURE TO A HIGH SALT DIET. J.A. McCaughran, Jr., R. Friedman*, and C.J. Juno*. Dept. Psychiatry and Behavioral Science, SUNY @ Stony Brook, New York, 11794.

The relationship between hypertension and the distribution of muscarinic and alpha adrenergic receptor sites in the heart of S/JR and R/JR rats was investigated. Four groups of S/JR and R/JR rats were used. The HLH groups were exposed to a high salt diet (8.0% NaCl w/w) *in utero* (via the pregnant dam), a low salt diet (0.4% NaCl w/w) during lactation, and a high salt diet after weaning. The HLL groups were exposed to a high salt diet *in utero* followed by low salt. The LLH groups were maintained on high salt after weaning and LLL groups were exposed to low salt throughout the study. Blood pressure (BP) and heart rate were monitored twice weekly to the termination of the study at 4 weeks after weaning. The antagonists [3H]-prazosin (PRAZ) and [3H]-quinuclidinylbenzilate (QNB) were used to assess the resepective density of alpha adrenergic and cholinergic receptor sites in the left and right ventricles (LV and RV), septum (SEP), and atria (AT). Although both HLH and LLH S/JR groups developed significant elevations in BP, the HLH group showed a facilitation in the postweaning development of salt-induced hypertension. No significant elevations in BP were noted in the R/JR groups. Both receptor sites were regionally distributed in the heart. QNB receptor sites were distributed as follows: AT > RV > SEP > LV. The inverse was found for the PRAZ binding sites: LV > SEP > RV > AT. With some exceptions, no signficant alterations in receptor site density were noted between the S/JR and R/JR groups, regardless of the prenatal or postweaning diet. However, a reduction in the density of PRAZ binding sites was found in the LV, and an increase in the density of PRAZ binding sites was found in the AT of the S/JR and R/JR HLH groups. Furthermore, all S/JR groups displayed a greater density of QNB sites in the AT than R/JR groups. The results indicate: (1) prenatal exposure to a high salt diet can facilitate the subsequent development of hypertension in genetically predisposed organisms; (2) the regional distribution of each receptor site corresponds to the degree of cholinergic or adrenergic innervation that the region receives; (3) *in utero* exposure to high salt has marked effects on the density of adrenoceptors in the LV and AT of the R/JR and S/JR groups and, therefore, may not be related to the pathogenesis of hypertension; and (4) the consistently greater density of atrial muscarinic receptors in the S/JR line suggests a disruption in parasympathetic innervation of this region. In summary, the data suggest an imbalance between sympathetic and parasympathetic input to myocardial tissue in the S/JR line. Moreover, in some cases this can be exacerbated by prenatal exposure to hypertensinogenic stimuli.

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CARDIOVASCULAR REGULATION: HYPERTENSION AND STRESS

208.1 AN ANALYSIS OF p-AMINOCLONIDINE BINDING IN RAT KIDNEY. B. Sripanidkulchai*, R. Dawson, S. Oparil* and J.M. Wyss (Spon: J. Beaton), Department of Anatomy and CVRTC, University of Alabama, Birmingham, AL 35294.

A population of α_2 adrenergic receptors are present in the kidney and they are increased in number in hypertensive models (Dahl and SHR). Previous studies using tritiated α_2 antagonists have indicated that there is a single binding site of these receptors. In this study the characteristics of these renal receptors have been examined in the SHR through the use of [^{3}H]-para-aminoclonidine (agonist; ^{3}H-PAC) binding.

Seven week old male SHR rats were killed by decapitation and the binding of ^{3}H-PAC (0.1-20nM) was examined in crude membrane preparations. Saturation analysis resulted in a curvilinear Scatchard plot which revealed two binding sites. The high affinity site had a Kd of 0.54nM and a Bmax of 47 fmoles/mg protein. The low affinity site had a Kd of 4.4nM and a Bmax of 132 fmoles/mg protein. Both the high (Kd=0.67nM) and low (Kd=6.8nM) affinity sites were also delineated by a second type of analysis (kinetic analysis; dissociation-association curves). Dissociation of ^{3}H-PAC was biphasic with apparent half-lives (50% dissociation) of approximately 72 seconds and 19 minutes respectively for the two sites.

A second study has addressed the questions of whether either of these two binding sites is different in seven week old SHR genetically hypertensive rats (n=5) versus their normotensive controls (WKY,n=5). The results demonstrate that the high affinity site displays significantly greater binding of ^{3}H-PAC at 0.5nM in the SHR. These two studies suggest 1) that two binding sites exist in the rat kidney (although it is possible that the difference reflects a single receptor with different affinity state), and 2) that the higher affinity site is altered in SHR.

208.2 ACTIVATION OF THE SYMPATHETIC NERVOUS SYSTEM IN ONE-KIDNEY PERINEPHRITIC HYPERTENSION IN THE RAT. R. M. Chinn, J. W. Manning and D. K. Hartle. Depts. of Physiology and Pharmacology, Emory University Medical School, Atlanta, Ga. 30322.

Factors maintaining elevated blood pressure (BP) in one-kidney perinephritic model of hypertension have not been studied in the rat. These experiments were designed to determine the involvement of the renin-angiotensin system (RAS), vasopressin system (VPS) and sympathetic nervous system (SNS) in this form of experimental hypertension. Groups of 300 g. male Sprague-Dawley rats underwent: (1) unilateral nephrectomy (UN), drinking tap water, (2) UN + contralateral perinephritic wrap, drinking tap water, (3) UN, drinking 0.9% saline, (4) UN + contralateral perinephritic wrap, drinking 0.9% saline.

The BPs of all animals were monitored chronically by means of a tail-cuff BP apparatus. When the animals became hypertensive (150 mmHg or above), they were anesthetized and a carotid catheter was inserted for direct BP measurements. Direct BP was recorded while the animals were conscious and unrestrained in their home cages on the day following catheterization. Control BPs were recorded for 30 minutes. The results indicate that no animals in groups 1 or 3 became hypertensive, indicating that reduced renal mass did not effect an increase in blood pressure in either sodium replete or sodium-loaded animals. A few animals in group 2 became hypertensive within one month of surgery but most animals remained normotensive. Saline-loading significantly enhanced the development of hypertension in perinephritic rats (4).

Each animal was then injected with saralasin (50 µg), a vasopressin antagonist (Peninsula Labs, #8109, 10 µg) and chlorisondamine (5 mg/kg). Fifteen minutes was allowed between drug treatments to allow maximal changes in BP to occur in response to each drug, but not enough time elapsed to allow reversal of the effectiveness of each agent.

BP reduction profiles during sequential drug blockade indicate that the RAS and VPS contributed 20 mmHg to support the BP of groups 1, 3 and 4 (no change in group 2). Ganglionic blockade with chlorisondamine significantly reduced BP in all animals. The decrease in blood pressure was much greater in the hypertensive animals in (2) and (4) than in the normotensive animals in groups 1-4. CONCLUSION: Sodium chloride accelerates the development of perinephritic hypertension. This form of hypertension is maintained primarily by an activation of the SNS. Supported by PHS-T35HL07305-06A1, 1F32HL06685-02, AHA, GA.

208.3 DORSAL RHIZOTOMY PARTIALLY PREVENTS ONE KIDNEY, ONE CLIP GOLDBLATT HYPERTENSION, N. Aboukarsh, S.R. Winternitz and J.M. Wyss, Department of Anatomy and CVRT, University of Alabama in Birmingham, Birmingham, AL 35294

Renal denervation can either diminish or prevent hypertension in several animal models of the disease. In most of these models it has been assumed that the renal efferent (sympathomotor) projection plays the critical role in hypertension. However, several recent studies have suggested that the renal afferent sensory) component is the important factor in at least one form of experimental hypertension, i.e. one and two kidney Goldblatt hypertension. In the present study, the contribution of this component was independently assessed, and the results indicate that intact renal sensory nerves are critical to the formation of 1 kidney, 1 clip Goldblatt hypertension. In twenty-two, male, Sprague-Dawley rats, unilateral right nephrectomies were performed at three weeks of age. The animals were then divided into three groups. In the first group (n=8), the dorsal root nerves through which the renal sensory nerves travel (thoracic 8-lumbar 2) were cut at four weeks of age. The two other groups served as sham controls. At five weeks of age a 300um silver clip was placed around the left renal artery of the animals in the group receiving the dorsal root lesions and one of the control groups (clipped control;n=9). The third group served as a non-clipped sham control (n=5). After one week the blood pressure of the clipped control animals had risen to hypertensive (>150mm Hg) levels and continued to rise for the following five weeks of the experiment (mean final blood pressure = 177mm Hg±14). In contrast the mean blood pressure in the lesioned group remained below 150mm Hg throughout the experiment. Mean blood pressure in this group 1 week after clipping was 140mm Hg ± 16 and the final weekly mean was 138mm Hg ± 20. It should be noted that although the lesion reduced the hypertensive effects, the lesioned group's mean blood pressure was significantly higher than the pressure of the non-clipped control (first week mean = 126 ± 9mm Hg; sixth week mean = 114 ± 11mm Hg). This study provides the first direct demonstration that renal afferent nerves are critical to the development of hypertension.

208.4 THE EFFECT OF DIETARY SODIUM INTAKE ON REFLEX TACHYCARDIA RESPONSES IN ONE-KIDNEY, ONE-WRAP RENAL HYPERTENSION. C. Hinojosa,* N.Ball* and J.R. Haywood. Pharmacology Dept. Univ. of TX Health Science Center, San Antonio, TX 78284.

Sodium depletion has been shown to prevent the onset of one-kidney, one-wrap renal hypertension while increased sodium intake enhanced the hypertensive process. The present study was designed to determine whether the permissive action of sodium in hypertensive rats was through a regulation of the activation of the sympathetic nervous system. Reflex increases in heart rate after atropine (0.4 mg/kg) were used as an index of increased sympathetic nervous system activity. Rats were maintained on low sodium (LS) (2 µEq/gm chow), normal sodium (NS) (100 µEq/gm chow) or high sodium (HS) (1000 µEq/gm chow) diet for two weeks prior to renal wrap or sham wrap procedure. Three days postwrap, animals were prepared with femoral artery and vein catheters under gaseous anesthesia. At least three hours later, the rats were treated with atropine (0.4 mg/kg) and reflex tachycardia responses to graded bolus injections of nitroprusside (5, 10 and 10 µg/kg) were determined. The relationship between the change in blood pressure and change in heart rate was determined for each animal, and the slope was taken as an index of baroreflex sensitivity. Administration of atropine caused a similar increase in heart rate in sham operated animals maintained on all three diets (+26±11 bpm LS, +32±6 bpm NS, and +40±6 bpm HS). In the renal wrapped animals, the response to atropine was proportional to sodium intake (+14±5 bpm LS, +44±4 bpm NS, and +84±10 bpm HS).

	Baroreflex Sensitivity	
	Sham	Wrap
LS	-0.62±0.14*	-0.63±0.18*
NS	-1.68±0.46	-1.63±0.31
HS	-1.57±0.34	-1.45±0.38

There were no differences in reflex function between renal wrapped and sham operated rats on any level of sodium intake. However, sodium depleted rats had a significantly reduced reflex sensitivity compared to animals maintained on normal or high sodium intake. These data support the hypothesis that prior sodium depletion prevented the increase in arterial pressure in one-kidney, one-wrap renal hypertension through an interference with the activation of sympathetic nervous system function. (Supported by NIH HL 26993 and American Heart Association, Texas Affiliate).

208.5 NOREPINEPHRINE AND SEROTONIN IN THE LOCUS COERULEUS OF THE DAHL SALT-SENSITIVE AND SALT-RESISTANT RAT. S.Y. Felten[1], D.L. Felten[1], and J.A. Weyhenmeyer[2], Depts. of Anat., Univ. Rochester Sch. Med., Rochester, NY 14642[1], and Sch. Life Sci., Univ. of Illinois, Urbana, IL 61801[2].

Dahl salt-sensitive (SS) and salt-resistant (SR) rats were raised on a low-salt diet (0.4%) or a high salt diet (8.0%), starting at 4 weeks of age for a period of 1 and 10 weeks, and were compared with standard Sprague-Dawley (SD) rats under the same dietary conditions. The SS rats raised on an 8% salt diet develop hypertension. The locus coeruleus (LC) was micropunched from the brains, and norepinephrine (NE), serotonin (5HT), and 5-hydroxyindoleacetic acid (5HIAA) were assayed with high performance liquid chromatography with electrochemical detection, based upon our past findings that LC develop premature dendritic length and branching in the SHR but not the normotensive WKY or Wistar control rats. After 1 week on 8% salt or 0.4% salt, no differences in NE levels were found, but after 10 weeks on 0.4% salt, NE levels were higher in SS rats than in SD rats, with SR rats at an intermediate level. NE levels may be genetically higher in LC in SS rats than in controls. Since there were no differences in LC levels across all 3 age groups after 10 weeks on 8% salt diet, NE levels in LC appear not to be causal to hypertension. After 1 week on an 0.4% salt diet, levels of 5HT were higher in SS than in SR or SD rats, while on an 8% salt diet for 1 week, 5HT levels in SS and SR rats were greater than in SD rats. In other words, 5HT levels rise only in SR rats after 1 week on a high salt diet. After 10 weeks on an 0.4% salt diet, 5HT levels were highest in SS, intermediate in SR, and lowest in SD rats (all significantly different), and were boosted on a high (8%) salt diet in all groups by approximately 2x but to the greatest extent in the SS rats (12.18 ± 2.72 ng mg protein). This highest level of 5HT, which responded to the high salt diet, correlates with the hypertensive state in these rats. The 5HIAA levels paralleled the 5HT levels and 5HT/5HIAA ratios did not differ significantly with high or low salt diets among the 3 groups, suggesting that turnover is not dramatically altered by the salt content in the diet. We suggest that the SS rats may demonstrate a genetic difference from their SR and SD controls in the development of the serotonergic system and its innervation of the locus coeruleus, and may show an acceleration of this difference when fed a high salt diet. Supported by NIH grant HL2 757.

208.6 EFFECT OF NEONATAL TREATMENT WITH MONOSODIUM GLUTAMATE ON THE ANTIHYPERTENSIVE ACTION OF CLONIDINE IN SPONTANEOUSLY HYPERTENSIVE RATS. A.R. Mosqueda-Garcia* and G. Kunos* (SPON: F.V. Abbott). Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec H3G 1Y6.

We have previously shown that a β-endorphin-like peptide is involved in the antihypertensive action of clonidine in spontaneously hypertensive rats (SHR) but not in normotensive rats. Outside of the pituitary, nerve cell bodies containing β-endorphin have only been demonstrated in the arcuate nucleus region (AN). Therefore, we tested the hypothesis that β-endorphin in the AN is involved in the antihypertensive action of clonidine. Monosodium glutamate (MSG) was used to selectively destroy the cell bodies of the AN and reduce brain β-endorphin levels.

SHR and Wistar Kyoto rats (WKY) received MSG during the neonatal period as reported (Nature, 278: 562, 1979). Systolic blood pressure (BP) and heart rate (HR) were measured by the tail cuff technique. At 3 months of age the developed hypertension of treated SHR (SHR-MSG) was similar to control animals (SHR-C). Basal BP was 170 ± 6 and 178 ± 4 mmHg for SHR-C and SHR-MSG respectively. Clonidine (5 ug/kg i.v.) reduced BP in both groups (SHR-C, -28 ± 5 and SHR-MSG,-17 ± 6 mmHg). The opioid antagonist, naltrexone (NTX, 2 mg/kg i.p.) was administered on a different day, 30 min prior to clonidine in the same animals. The response to clonidine was inhibited by NTX in SHR-C but not in SHR-MSG animals (SHR-C,-8 ± 4; SHR-MSG,-20 ± 6 mmHg). Similar data were obtained for HR. NTX was without effect in untreated WKY and the response to clonidine or NTX was not modified by MSG-treatment of these animals.

The absence of an effect by NTX in SHR-MSG animals suggests that the lesioning of the AN damages of a β-endorphin system which is involved in the antihypertensive action of clonidine. Moreover the MSG-treatment has no significant effect on this response in WKY, in which the opioid contribution to this effect is not normally detectable.

208.7 CENTRAL NERVOUS SYSTEM CARDIOVASCULAR TOXICITY OF SUBCUTANEOUS MONOSODIUM GLUTAMATE TREATMENT IN THE RAT. J. W. Manning and D. K. Hartle. Depts. of Physiology and Pharmacology, Emory University School of Medicine, Atlanta, GA. 30322.

Straight chain amino acids such as glutamic and aspartic are potent neuroexcitotoxins (see Olney, Neurosci. Res. Prog. Bull., V.19, No. 4). Peripheral administration of these compounds causes significant central neural toxicity and cell death in regions of the brain that are not protected by the blood-brain barrier (BBB). Brain damage in adult rats is largely confined to circumventricular organs (highly vascularized regions of the central nervous system without a BBB). More extensive damage is produced if animals are treated neonatally, e.g., monosodium glutamate (MSG) causes depletion of cell bodies in the median eminence and in the surrounding arcuate nucleus.

The circumventricular organs are very important sites of integration of humoral and neural afferent cardiovascular information. Humoral substances, as angiotensin II (AII), for instance, interact at circumventricular organs with neuronal substrates and promote central neural responses. AII causes a centrally-mediated increase in blood pressure due sympathetic activation and augmented vasopressin secretion. AII also causes thirst and induction of drinking behavior. These studies were designed to test whether pretreatment of rats with sublethal subcutaneous injections of MSG would affect the central actions of AII. Additionally, the effects of MSG treatment on blood pressure in spontaneously hypertensive rat was tested.

The results indicate that 4 day old rats treated with MSG showed a dose dependent decrease in drinking response to peripherally administered AII when they were tested as young adults. MSG produces drinking and pressor deficits to AII in some, but not all, adult rats treated with MSG. MSG treatment lowered mean blood pressure from 220 mmHg to 120 mmHg in a group of spontaneously hypertensive rats but had little effect on blood pressure in normotensive rats. The decrease in blood pressure was sustained in 1/3 of the hypertensive animals while 2/3 of these animals were again hypertensive 24 hrs after treatment. These results suggest that MSG treatment causes significant toxicity (sometimes reversibly, sometimes irreversibly) to central neural mechanisms that are involved in blood pressure regulation and fluid balance. Neural structures within or close to circumventricular organs appear to play an important role in maintenance of high blood pressure in the spontaneously hypersive rat. PHS-532HL06685-02, AHA, GA & Emory University.

208.8 CONTRIBUTION OF VASOPRESSIN TO HYPERTENSION AFTER SOLITARY TRACT LESIONS OR SINOAORTIC DENERVATION PLUS VAGOTOMY IN THE DOG. D.B. Averill, K.L. Barnes and C.M. Ferrario. Research Division, Cleveland Clinic Foundation, Cleveland, OH 44106.

The mechanisms responsible for the hypertension which follows central interruption of vagal and carotid sinus afferents in the dog are not well understood. In this study we interrupted the vagal and carotid sinus afferents centrally in halothane anesthetized mongrel dogs by lesioning the solitary tract (TS) close to the entrance of the afferents into the medulla. The TS on both sides of the brain stem was located with vagal evoked potentials and lesioned in 5 dogs; two animals received sham TS lesions. To compare the effects of peripheral interruption of these afferents to the effects of TS lesions, six dogs were studied after bilateral vagotomy and electrophysiological identification and section of both carotid sinus nerves (SAVD). The effectiveness of both procedures was confirmed pharmacologically by the absence of reflex bradycardia after phenylephrine (20 μg/kg, i.v.). Bilateral interruption of the rostral TS was verified histologically in all 5 dogs in the TS lesion group.

Blood pressure rose rapidly in both TS lesioned and SAVD dogs after anesthesia was discontinued. Two hours following baroreceptor denervation mean aortic pressure (MAP) and heart rate were 165 ± 21 mmHg and 172 ± 17 beats/min in the TS lesioned dogs and 163 ± 9 mmHg and 196 ± 8 beats/min in the SAVD animals. Administration of a vasopressin antagonist [$d(CH_2)_5$ Tyr (Me) arginine vasopressin, 20 μg/kg, i.v.] decreased MAP in both TS lesioned (-25 ± 2 mmHg, $p < 0.001$) and SAVD animals (-34 ± 5 mmHg, $p < 0.002$) but did not affect MAP in two sham lesioned dogs (-4 ± 5 mmHg, n.s.). Thirty minutes following administration of the vasopressin antagonist MAP was the same in the three groups of dogs (TS lesion: 141 ± 24; sham lesion: 139 ± 10; SAVD: 141 ± 7 mm Hg). Subsequent administration of hexamethonium chloride (15 mg/kg, i.v.) reduced MAP by a comparable amount in each group of dogs.

This study indicates that either central or peripheral interruption of baroreceptor, chemoreceptor and pulmonary stretch receptor afferent fibers produces an acute hypertension which is primarily dependent upon an increase in circulating vasopressin. (Supported in part by grants from NHLBI, HL-6835 and HL-31256).

208.9 CARDIOVASCULAR AND NEUROENDOCRINE RESPONSES DURING ACUTE BEHAVIORAL STRESS IN RATS WITH NTS LESIONS. R.A. BUCHHOLZ, J.W. HUBBARD, M.A. NATHAN, T.K. KEETON*. Dept. of Pharmacology, Univ. of Texas Health Science Center, San Antonio, Texas 78284.

In this study, we evaluated the cardiovascular and neuroendocrine responses of rats at rest and during behavioral stress 3-4 weeks after placement of electrolytic lesions in the NTS. Means and standard deviations (SD), an index of lability, were computed for MAP and HR during a 1 hr baseline recording period and during a single 30 min signalled avoidance/escape conditioning session. Plasma concentrations of epinephrine (EPI) and norepinephrine (NE), and plasma renin activity (PRA) were measured at the end of the rest and stress periods.

	MAP (mm Hg)		SD MAP (mm Hg)		HR (bpm)	
	Rest	Stress	Rest	Stress	Rest	Stress
NTS(7)	121±3	166±3*,**	29.2±2.1**	24.9±1.8**	384±11	481±8*,**
CON(4)	116±1	133±4*	5.9±1.1	8.7±1.4	356±17	443±17*

* p<0.05 from Rest
**p<0.05 from Control

	EPI (pg/ml)		NE (pg/ml)		PRA (ng/ml/hr)	
	Rest	Stress	Rest	Stress	Rest	Stress
NTS(7)	46±8	465±68*	210±24	617±59*,**	5.0±0.8**	6.7±1.6**
CON(4)	39±14	344±21*	209±9	321±47	7.9±0.8	28.9±9.6

* p<0.05 from Rest
**p<0.05 from Control

No differences were found at rest between NTS lesion and control rats for MAP, HR, EPI, or NE. NTS lesion rats showed significantly greater lability of the MAP and a lower resting PRA than control rats. Both NTS lesion and control rats exhibited a significant increase in MAP and HR during behavioral stress. The increase in MAP for NTS lesion rats was nearly 3 times that of controls. The increase in MAP in NTS lesion rats was accompanied by increases in plasma NE and EPI with no change in PRA. Control rats also showed an increase in plasma EPI levels during stress that was not different from NTS lesion rats. PRA in control rats was significantly higher than that of NTS lesion rats during stress. These results indicate that rats with NTS lesions exhibit enhanced MAP responses during stress that are supported primarily by an augmented sympathetic discharge to the vasculature. Plasma renin activity may be suppressed during stress in rats with NTS lesions by a direct action of the high MAP on the kidney. Supported by NIH HL27046, HL24529, and AHA, Texas Affiliate.

208.10 FEED-STARVE CYCLING IN DIETARY OBESITY INDUCES MODERATE HYPERTENSION VIA ALTERATIONS IN THE AUTONOMIC REGULATION OF CARDIOVASCULAR FUNCTION D.O. Nelson and P. Ernsberger, Neuroscience Program and Physiology Dept., Northwestern University, Chicago, IL 60611.

In hypertension associated with human obesity, blood pressure (BP) is best related to rates of change in weight rather than to static obesity. Accordingly, BP and heart rate (HR) were determined by tail cuff during cycles of overfeeding and starvation. Dietary obesity was produced in 19 rats by feeding fortified (AIN 76) sweet milk in addition to regular chow, while 10 rats continued to receive chow only. Na^+ intake was equalized to the control level of approximately 1.4 mmol/day. Caloric intake in the sweet-milk group increased from a baseline of 114 ± 4 kcal/day to 150 ± 3, accompanied by tachycardia (391 ± 7 bpm vs. 354 ± 10) within two weeks after the start of the diet, prior to significant excess weight gain, but BP was unchanged. Four weeks after the initiation of the diet, half of the obese rats were fasted for 4 days, receiving only mineral and vitamin solution (providing 1.4 mmol Na^+) and tap water. After 4 days of fasting BP fell from 126 ± 3 mmHg to 106 ± 4, and HR fell 25 ± 9 bpm. BP returned to baseline after 2 days of refeeding and continued to rise, reaching near-hypertensive levels (142 ± 2 mmHg) by the 12th day. HR remained depressed through 2 days of refeeding, but the prefasting tachycardia returned by the end of the 12-day refeeding period. BP and HR fell during each subsequent fast, returning to elevated prefast levels with refeeding. At the end of the 3rd refeeding period, the rats were anesthetized and fitted with femoral arterial and venous catheters. Hypertension and tachycardia persisted in the cycled animals, while uncycled obese rats remained normotensive. In both groups the HR decrease after beta blockade (metopolol, 1 mg/kg) was enhanced and the HR increase after N-methylscopolamine (2 mg/kg) was eliminated, suggesting increased sympathetic and decreased para- sympathetic tone. Cardiac beta-receptor binding, as measured with ^{3}H-dihydroalprenolol, was down-regulated (control B_{max} (fmol/mg protein): 67 ± 6, sweet-milk: 37 ± 3, cycled: 47 ± 3), consistent with increased sympathetic activity, while muscarinic binding (^{3}H-N-methylscopolamine) was unchanged. Thus feed-starve cycling, but not static obesity, induces hypertension associated with increased sympathetic tone, and is a possible model for human obese hypertension. Initial results suggest that the paraventricular nucleus mediates the effects of feed-starve cycles on cardiovascular function.

208.11 PERIPHERAL ADRENERGIC MECHANISMS IN REPEATED IMMOBILIZATION STRESS IN RATS. Z.Zukowska-Grojec*, J.Culman* and I.J. Kopin (SPON: E.Stadlan). Laboratory of Clinical Science, NIMH, Bethesda MD 20205.

Both central and peripheral neurons are involved in mechanisms of adaptation of the cardiovascular system to chronic stress. In the present study we investigated the effects of repeated immobilization (1-7 x IMO, 2 hrs daily) on pressor and catecholamine (CA) responses to stimulation (ST, 0.1 and 1.0 Hz, 50 V) of sympathetic outflow from the spinal cord or administered norepinephrine (NE, 0.1 and 1.0 μg/kg, i.v.) in pithed, vagotomized rats, intact or after bilateral adrenal demedullation (MEDX). When conscious, tail-artery cannulated rats were IMO for the first time (1xIMO) there were increases in blood pressure (BP, +23±5 mm Hg), plasma NE (+1.3 ± 0.1 ng/ml) and epinephrine (EPI, +2.1 ± 0.5 ng/ml). After 1xIMO, basal BP and plasma NE of pithed rats increased whereas pressor responses to ST (0.1 Hz) and NE (0.1 μg/kg) were lower than in pithed non-stressed rats. Those differences disappeared after desipramine (DMI, 0.3 mg/kg, i.v.). MEDX 1 x IMO rats showed lesser BP (50%) and plasma NE (30%) responses than intact 1 x IMO rats but pressor responses to ST and NE remained reduced accompanied by decreased NE response to ST. Those differences persisted after DMI, suggesting that decreased removal of adrenal CA (↓ uptake) may be responsible for the elevated CA levels in the intact rats. Seventh IMO (7xIMO) caused less increment in BP (+19 ± 2 mm Hg) and plasma NE and EPI (+0.8 ± 0.1 and +1.1 ± 0.1 ng/ml, respectively) than in 1 x IMO rats. In pithed 7 x IMO rats, basal BP returned to control values while basal plasma CA levels remained elevated. Pressor responses to 0.1 Hz ST were reduced but normalized in the presence of DMI.

In conclusion, activation of the sympatho-adrenomedullary system and vascular responsiveness to CA diminish with repeated stress. Reduced pressor responses of once-stressed rats are not due to decreased CA release from sympathetic neurones or adrenal medulla, nor do they seem to result from receptor changes (Torda et al., J Pharm Exp Ther 216, 334, 1981) which occur later after 7 x IMO. There does, however, appear to be a transient reduction in vascular reactivity after 1 x IMO.

208.12 CARDIOVASCULAR AND SOMATOMOTOPIC RESPONSES TO STARTLING SENSORY STIMULI IN SPONTANEOUSLY HYPERTENSIVE RATS. R. Rettig, M. Geyer, M.P. Printz*. Univ. of California, San Diego, School of Medicine, La Jolla, CA 92093.

Primary hypertension has been described as being closely linked with cardiovascular and behavioral hyperresponsivity to a variety of environmental stimuli and such hyperresponsivity has been implicated in the pathogenesis of sustained hypertension. To test whether spontaneously hypertensive rats (SHR) are hyperresponsive - in terms of their cardiovascular and behavioral reactions - to a well-defined sensory stimulus, when compared to the normotensive Wistar-Kyoto control strain (WKY), adult male rats of both strains were subjected to 15 consecutive trials of either tactile (air puff to the back of the animal, 15 p.s.i., 40 ms, n = 8 per group) or acoustic (123 dB, 4 kHz, 40 ms, n = 8 per group) sensory stimuli with an intertrial interval of 60 s. For application of the stimuli and simultaneous measurement of the somatomotoric and cardiovascular responses, rats were placed into a suspended plexiglass cylinder. Mean arterial pressure and heart rate were measured continuously via an indwelling catheter from the abdominal aorta and displayed on a chart recorder. The magnitude and latency of the somatomotoric startle responses was recorded as displacement of the cylinder by a ceramic phonograph cartridge and the data were analyzed by computer (one reading per ms for 200 ms, starting with the onset of the stimulus). SHR exhibited significantly greater maximal and average motor responses as well as a shorter latency for the maximal response following both tactile and acoustic stimulation. Heart rate and pressor responses were also significantly increased in SHR. However, whereas the magnitude of the heart rate responses remained about the same within each strain and was consistently elevated in SHR throughout the entire trial sessions, pressor responses decreased in both strains towards the end of the trial sessions.

Taken together, our data indicate that SHR exhibit cardiovascular and behavioral hyperresponsivity to acute sensory stimulation. The data are in keeping with the hypothesis that increased transient pressor responses occurring repeatedly during daily life situations in hypertensive subjects contribute to the development and/or maintenance of hypertension. (Supported in part by HL25457, SCOR Hypertension.)

208.13 HYPERRESPONSIVENESS OF CENTRAL AND PERIPHERAL MONOAMINERGIC MECHANISMS TO COLD STRESS IN 4 WEEK DOCA/NaCl HYPERTENSIVE RATS. Y.F. Chen, S. Nagahama*, S.R. Winternitz and S. Oparil*. Cardiovascular Research and Training Center, University of Alabama in Birmingham, Birmingham, AL 35294.

Previous studies from our laboratory failed to provide evidence for increased activity of the sympathetic nervous system (SNS) in conscious, unrestrained deoxycorticosterone-NaCl (DOCA/NaCl) hypertensive rats (3 and 3 weeks after DOCA/NaCl treatment) studied in the resting state. To examine the hypothesis that basal SNS activity and the responsiveness of SNS to stress are enhanced in DOCA/NaCl rats with established hypertension, plasma norepinephrine (NE), epinephrine (E) and prolactin (PRL) were measured in conscious, unrestrained 4 week DOCA/NaCl hypertensive and control (uninephrectomized) rats before and after cold exposure (4 hours, 4°C).

Systolic blood pressure measured by the tail cuff method in DOCA/NaCl rats was significantly greater than in H_2O controls [181±5(n=14) vs 128±5(n=11)mmHg, P<0.01] at 4 weeks. Results [Mean±SEM] of plasma NE(pg/ml), E(pg/ml) and PRL(ng/ml) were:

	Before Cold Stress			After Cold Stress		
	NE	E	PRL	NE	E	PRL
H_2O	328±34	276±44	17±1	1068±64*	404±55	18±1
DOCA	550±56#	496±59#	32±3#	1658±124#*	834±160#	18±1

# P<0.05, as compared to H_2O controls
* P<0.05, as compared to before stress value.

These results demonstrate that 4 week DOCA/NaCl treated rats have increased circulating levels of NE and E both in the resting state and following cold stress. Basal plasma PRL levels were elevated in DOCA/NaCl animals compared to control, suggesting reduced hypothalamic dopaminergic activity. Following cold stress, plasma PRL levels fell in the DOCA/NaCl rats, suggesting that central dopaminergic activity was increased. In contrast, no stress related reduction in plasma PRL was seen in control rats. Taken together, these data support the concept that DOCA/NaCl hypertensive rats have increased sympathetic tone which may be related to reduced hypothalamic dopaminergic activity.

208.14 ENHANCED RENAL SYMPATHETIC NERVE RESPONSE TO STRESS IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS (SHR) ON HIGH SODIUM DIET. J.P. Koepke* & G.F. DiBona* (SPON: E.M. Burns). Dept. Int. Med., Univ. Ia. Col. Med. & VAMC, Iowa City, IA 52242.

The effects of stressful environmental stimulation (air jet to head) on renal sympathetic nerve activity (RSNA), urine flow rate (V), sodium excretion ($U_{Na}V$), glomerular filtration rate (C_{IN}) and renal plasma flow (C_{PAH}) were examined in conscious SHR (MAP=150 mmHg) and normotensive Wistar-Kyoto rats (WKY, MAP=115 mmHg) on either normal (NNa) or high (HNa; 0.9% NaCl to drink for 15 days) sodium diets. 24 hours prior to experimentation, an electrode was implanted around a renal nerve branch for recording RSNA. A different group had a urinary bladder catheter implanted for urine collections.

Data for RSNA (integrated voltage) are mean±SE integrator resets/min; C=Control, R=Recovery; $p < .05$ vs. C.

	SHR-NNa n=6	SHR-HNa n=6	WKY-NNa n=6	WKY-HNa n=5
C	6.2±0.6	5.9±0.4	7.0±0.6	7.0±0.8
Air	10.9±1.0*	13.6±1.0*	7.7±0.6	7.0±1.1
R	6.2±0.5	7.5±0.5	6.8±0.5	6.6±0.7

Renal function data for SHR on NNa and HNa diets are mean±SE per 100g BW:

	V μl/min	$U_{Na}V$ μEq/min	C_{IN} ml/min	C_{PAH} ml/min
SHR C	15.7±2.5	2.5±0.6	0.87±0.05	4.34±0.47
NNa Air	14.4±3.2	1.8±0.6*	0.86±0.05	4.29±0.49
(8) R	15.6±3.0	2.7±0.7	0.90±0.05	4.37±0.56
SHR C	27.1±5.0	4.3±0.8	0.89±0.06	3.78±0.32
HNa Air	17.1±3.3*	2.5±0.5*	0.77±0.08	2.94±0.19
(8) R	25.1±3.4	4.1±0.7	0.83±0.07	3.15±0.31

In SHR, HNa augmented the RSNA and antinatriuretic responses to air stress ($p < .05$). Air stress did not affect RSNA or renal excretion in WKY on NNa or HNa diets.

Conclusion: The enhanced RSNA and antinatriuretic responses to air stress in SHR on HNa diet may reflect a sodium-dependent facilitation of central neurotransmission. In SHR, the antinatriuretic response to air stress is dependent on an increased tubular sodium reabsorption (NNa) and a renal vasoconstriction with decreased filtered sodium load (HNa). The greater renal responses in SHR than WKY may reflect a genetic predisposition to increase RSNA during stressful environmental stimulation. (NIH AM 15843, HL 14388 & VA).

208.15 EFFECTS OF BIOBEHAVIORALLY-ASSISTED RELAXATION TRAINING ON BLOOD PRESSURE AND PLASMA RENIN, ALDOSTERONE AND CORTISOL IN ESSENTIAL HYPERTENSIVES. J.W. Turner, Jr.*, A. McGrady, T. Fine*, M. Woerner* and J. Higgins*, Dept. of Physiology and Behavioral Med. Clinic, Medical College of Ohio, Toledo, Ohio 43699

The present study examines the effect of relaxation training assisted by flotation REST (Restricted Environmental Stimulation Therapy) or EMG biofeedback on blood pressure (BP) and plasma renin, aldosterone and cortisol in 18 unmedicated subjects with uncomplicated essential hypertension. The average age was 45 and beginning BP averaged 148/92. The experimental design included a 6 week baseline and multiple blood samples per sampling session. Each subject experienced 20 treatment sessions, one hour each, of REST or EMG biofeedback over a 10 week period and a 12 week follow-up. The REST environment consisted of a 4' x 8' rectangular chamber, completely enclosed and filled to a 10-inch depth with saturated $MgSO_4$ solution (sp. gr. 1.28) maintained at 94 F. The supinely floating subject experienced minimal light, sound, temperature awareness and spatial orientation. The biofeedback group (n=12) received forehead EMG feedback. Each treatment session included relaxation training via modified autogenic phrases which were practiced daily at home. In both groups systolic and diastolic BP, plasma renin, aldosterone and cortisol all decreased significantly ($p < .01$, two way ANOVA) from baseline to post-treatment. In both REST and biofeedback subjects 67% achieved clinically significant BP reductions. However, 33% of REST subjects who showed significantly decreased renin, aldosterone and cortisol did not significantly reduce BP and 25% of biofeedback subjects who achieved clinically significant BP reductions did not show decreased hormone levels. The results of this study demonstrate that REST-assisted and EMG biofeedback-assisted relaxation training are associated with decreased plasma renin, aldosterone and cortisol and clinical significant decreases in BP. It appears that changes in BP and changes in the levels of renin, aldosterone and cortisol can be independent. This research supported in part by BRS #94367 - Medical College of Ohio.

208.16 PROLONGED ENHANCEMENT OF INTRASPINAL SYMPATHETIC TRANSMISSION BY BRIEF PERIODS OF LOW-FREQUENCY ACTIVATION. Ralph L. Myers* and Donald N. Franz (SPON: J.H. Petajan). Department of Pharmacology, Univ. of Utah School of Medicine, Salt Lake City, Utah 84132.

Brief periods of repetitive (tetanic) stimulation of descending excitatory sympathetic pathways in the cervical spinal cord produces long-term potentiation (LTP) of subsequent transmission to sympathetic preganglionic neurons (SPGNs) that subsides in 20-30 min. Tetanic stimulation at 50 Hz for 10 sec repeated four times at 20 min intervals also produces incremental increases in steady-state transmission to SPGNs that are sustained for longer than 3 hr (Soc. Neurosci. Abstr. 9:543, 1983). The present study was conducted to determine whether such prolonged increases could be produced by stimulation at lower, more physiological frequencies of sympathetic activity.

Sympathetic discharges recorded from upper thoracic preganglionic rami were evoked by stimulation of descending excitatory pathways in the cervical dorsolateral funiculus of unanesthetized, spinal cats at 0.1 Hz. Tetanic stimulation at 10 Hz for 20 or 30 sec produced significant LTP (130-180% of control) that was less than produced by stimulation at 50 Hz for 10 sec. Stimulation at 10 Hz for 30 sec repeated four times at 10 or 20 min intervals produced incremental increases in steady-state transmission that frequently reached 200% of control levels or higher and were sustained for more than 6 hr. Four tetanizations of 50 Hz for 10 sec at 10 or 20 min intervals produced similar increases in steady-state transmission. However, no increases were produced at either frequency when intervals of tetanic stimulation were 60 min.

The demonstration that brief periods of repeated activation of descending sympathetic pathways to SPGNs at 10 Hz can produce prolonged periods of increased transmission suggests that similar increases may occur in response to physiological stimuli. Such increases may contribute to the development of hypertension during emotional stress or other neurogenic conditions. (Supported by HL-24085, GM-07579, & Utah Heart. Assn.)

208.17 THE EFFECTS OF CALCIUM CHANNEL BLOCKERS ON VASCULAR ADRENERGIC NEUROTRANSMISSION IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND WISTAR KYOTO RATS (WKY). W.H. Cline, Jr.. Dept. of Pharmacol., So. Ill. Univ. Sch. of Med., Spfld., IL 62708

Altered adrenergic neurotransmission has been reported for several vascular beds of SHR, both in isolated, perfused in vitro vascular preparations and in the in situ, blood-perfused mesenteric vascular bed. In adult, male SHR, an enhanced responsiveness of presynaptic beta-adrenergic and angiotensin II receptor-mediated facilitation of mesenteric vascular adrenergic neurotransmission has been demonstrated in vivo. No difference was observed in the sensitivity of presynaptic alpha-adrenergic receptor-mediated auto-inhibition of adrenergic neurotransmission in the mesenteric vascular bed of adult SHR in vivo. The effects of two chemically different calcium channel blockers on mesenteric vascular adrenergic neurotransmission were studied in 13 to 16 week old, male SHR and age-matched, normotensive WKY control rats. The in situ, blood-perfused mesenteric vascular bed was employed in these studies. Nifedipine (N), administered i.a. at 300 ng/Kg, caused a significant shift to the right of the mesenteric perfusion pressure frequency-response curves to periarterial nerve stimulation (PNS) and a significant shift to the right of the mesenteric perfusion pressure dose-response curves to exogenous norepinephrine (NE). Since the effect on the PNS response curves was similar in magnitude to the effect on the NE response curves, a postjunctional site of action is suggested. N, at 100 ng/Kg, shifted the PNS mesenteric perfusion pressure response curve to the left in SHR and to the right in WKY, with the difference being significant. This dose of N had no significant effect of the mesenteric perfusion pressure response curves to NE. Verapamil (V), administered i.a. at 300 ng/Kg, caused a significant shift to the left of the mesenteric perfusion pressure curves in response to PNS which did not differ between SHR and WKY. V did not alter the mesenteric perfusion pressure response curves in response to NE. Thus, calcium channel blockers may either facilitate vascular adrenergic neurotransmission or inhibit postjunctional responses to NE depending on the specific agent and/or dose administered. These variable and seemingly paradoxical effects may be the result of the reported alpha-2 adrenergic receptor blocking properties of these agents, especially that of V. The differential effect of N on the PNS responses of SHR and WKY indicates altered calcium-dependent mechanisms or receptor sensitivity (alpha) in the SHR. (Supported by Am. Heart Assoc./Ill. Affiliate.)

208.18 DIHYDROERGOTOXINE DECREASES BLOOD PRESSURE IN SPONTANEOUSLY HYPERTENSIVE RATS BY INTERACTING WITH PERIPHERAL DOPAMINE RECEPTORS. G. Sagheddu*,C.Missale, P.Liberini*, L.Castelletti*, G.Picotti*, M.O.Carruba*, M.Memo and P.F.Spano.(SPON: E. Parati). Inst.Pharmacol.Exp.Ther., Univ.Brescia and Inst. Pharmacol. Univ.Milan, Italy.

Different dopamine (DA) receptors affecting the cardiovascular system have been identified in peripheral nervous system. In the present study we evaluated the effect of the DAergic ergot derivative dihydroergotoxine (DHT) on the cardiovascular system of normotensive and spontaneously hypertensive (SH) rats.

DHT (10 ug/Kg s.c.) decreases mean carotid blood pressure in uretane-anesthetized SH rats but failed to modify the same parameters in normotensive rats. The effect was statistically significant 15 min after the injection and relatively long lasting (up to 90 min). The calculated ED_{50} was 2.5 ug/Kg. A marked decrease of plasma circulating norepinephrine levels was also detected at the time when DHT induced the reduction in blood pressure.

Pharmacological characterization of the phenomenon indicated that this action is mediated by stimulation of DA receptors since pretreatment with haloperidol,cis-flupentixol but not trans-flupentixol completely prevent the reduction in blood pressure induced by DHT. Moreover, in spontaneously hypertensive rats pretreated with domperidon, (-)sulpiride but not (+)sulpiride a challenge dose of DHT did not reduce mean blood pressure values.

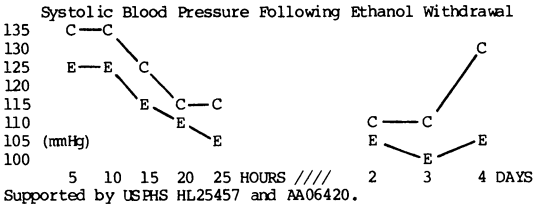
These results suggest that the DAergic ergot derivative DHT induces modifications in the cardiovascular system of SH rats by interacting with peripheral DA receptors which are possibly located on the terminals of sympathetic neurons.

208.19 REDUCED BLOOD PRESSURE, PRESSOR RESPONSES, AND APPETITE SUBSEQUENT TO ESTRADIOL TREATMENT. T.A. McCaffrey*, J.A. Czaja, and E.A. Baronowsky* (SPON: L.J. Pellegrino). Dept. of Psych. Sciences, Purdue Univ., W. Lafayette, IN 47907.

Using the guinea pig as our animal model, we have attempted to document the effects of gonadal steroids on the cardiovascular system, and the relationship of these changes to concurrent behavioral responses of conscious, unrestrained animals. Initially, we found that a single 30 μg estradiol injection was sufficient to produce significant and prolonged depression in blood pressure with a latency of 12 - 24 hours, and that these cardiovascular changes were significantly correlated with concurrent changes in water intake (McCaffrey & Czaja, Neurosci. Abst., 9, 544, 1983). The present experiment was designed to evaluate these relationships in males. Estradiol stimulation has previously been found to produce a number of effects in male guinea pigs (GPs) which were qualitatively similar to effects of this hormone in female GPs (Czaja, Phys. Behav., 33, in press, 1984). Twenty male guinea pigs, half of which had been castrated two weeks previously, were housed in metabolic cages and catheterized via the carotid artery and jugular vein. Baseline measurements were followed by four days of treatment with either 3 μg of estradiol benzoate (EB, N=5 intact, N=5 castrate) or the oil vehicle. Among the variables measured daily were food and water intake, body weight, urine and fecal output, blood pressure, heart rate, pressor responses to infusions of 1.6 μg norepinephrine (NE), and hematocrit. Since no significant differences were found in the responses of intact and castrated males, their data were combined for analysis. Estradiol treatment resulted in significant decreases in resting systolic and diastolic blood pressure (df=18, t=5.40, $p<.001$ and t=4.70, $p<.001$, respectively) and in pressor responses to $\overline{NE}$ (t=3.77, $p<.01$ and t=3.98, $p<.001$). Neither heart rate nor $\overline{NE}$ induced bradycardia were significantly affected by estradiol. Food intake, but not water intake, was also significantly depressed by the estradiol treatment. No significant correlations were found between the degree of depression in the ingestive behaviors and the cardiovascular changes. Thus, the present results confirm that relatively low doses of estradiol can effectively lower blood pressure and cardiovascular responsiveness in the conscious, unrestrained guinea pig. However, there was no evidence that these cardiovascular changes are coupled to the observed changes in ingestive behavior.

208.20 RAT BLOOD PRESSURE DURING ETHANOL WITHDRAWAL. L.Y.Koda, S.G.Madamba* and F.E.Bloom. Alcohol Research Center, Research Institute Scripps Clinic, La Jolla, CA 92037.

Recent clinical studies indicate a positive correlation between high ethanol consumption and hypertension. Since acute ethanol withdrawal may precipitate CNS hyperexcitability we have examined the possibility that acute ethanol withdrawal may also precipitate a cardiovascular dysfunction. Rat blood pressure and heart rate were monitored in conscious rats during ethanol withdrawal. Sprague Dawley rats with free access to food and water were placed in plexiglass chambers and exposed for 3 weeks to either ethanol vapor (E) or to air alone (C). Blood ethanol levels in the ethanol treated group were maintained above 100 mg/dl during the last week of ethanol exposure. Four to 18 hours prior to blood pressure measurement, rats were removed from the ethanol chambers, anesthetized with halothane and implanted with an indwelling abdominal aortic cannula. Rats were either placed overnight in the plexiglass chambers and re-exposed to ethanol or air or withdrawn from ethanol. Blood pressure and heart rate were monitored at various periods from 5 hours to 4 days following the cessation of ethanol exposure. Analysis of variance revealed a significant decrease in blood pressure and heart rate in rats exposed to ethanol vapor as compared to controls. There was a significant negative correlation between the average blood ethanol levels, taken during ethanol exposure, and systolic blood pressure at 5 hours, 2 days and 3 days following cessation of ethanol exposure. Although rats exposed to ethanol weighed less than control rats, hypotension was probably independent of this weight difference since blood pressure of weight-matched control rats did not differ significantly. These results indicate that although a cardiovascular dysfunction (hypotension) exists, ethanol associated hypertension is not due solely to ethanol withdrawal.



Supported by USPHS HL25457 and AA06420.

208.21 EFFECTS OF PROPRANOLOL WITH OR WITHOUT NALOXONE IN THE ISOLATED PERFUSED RAT HEART DURING MYOCARDIAL ISCHEMIA AND REPERFUSION. A.Y.S. Lee*, C.Y. Zhan* and T.M. Wong* (SPON: J.C. Hwang). Department of Physiology, University of Hong Kong, Hong Kong.

The effects of propranolol with or without naloxone on the electrical activities, left ventricular systolic and diastolic pressures and heart rate following myocardial ischemia and reperfusion were studied in isolated perfused rat heart. The left ventricular pressures and heart rate were greatly reduced and cardiac arrhythmias invariably occurred during myocardial ischemia and reperfusion. These effects of myocardial ischemia and reperfusion were attenuated with prior administration of propranolol (20 ug) into the isolated heart. With pretreatment of both naloxone (200 ug) and propranolol (20 ug), the cardiac arrhythmias were more markedly attenuated and both left ventricular pressures and heart rate were restored to or above the original levels during the reperfusion period.

When fibrillation was induced by myocardial ischemia and reperfusion, propranolol (20 and 200 ug) attenuated the arrhythmias dose-dependently. Administration of both propranolol (20 ug) and naloxone (200 ug) apparently attenuated the arrhythmias more markedly than propranolol alone.

The results are compatible with the findings that myocardial ischemia and reperfusion induce an increased release of both catecholamines and endogenous opioid peptides which cause alterations in cardiac functions resulting in arrhythmias and reduction in contractility and excitability of the heart. Joint administration of both propranolol and naloxone appears to be a more effective remedy of myocardial ischemia and reperfusion. These results could be of clinical implications in the prevention and treatment of ischemic heart diseases. (Supported by Hong Kong University Research Grant 335/034/0008 and Wing Lung Medical Research Fund 311/030/8009/64 to T.M.W. and China Medical Board Fellowship to C.Y.Z. from Zhongshan Medical College, China).

208.22 ALTERATIONS IN Ca^{++}/Mg^{++} ATPase IN BRAINS OF SHR/WKY RATS. D.H. Ross and H.L. Cardenas*. Division of Molecular Pharmacology, The University of Texas Health Science Center, San Antonio, TX 78284.

Recent studies have reported that calcium metabolism in spontaneously hypertensive rats (SHR) and their normotensive controls (WKY) is significantly different. Ca^{++} binding to high affinity sites on plasma membranes from heart and liver, nerve endings and erythrocytes of SHR exhibited reduced binding capacity, compared to WKY controls. Ca^{++} transport in subcellular membrane fractions of brain is also altered. Ca^{++} uptake into mitochondria was increased in SHR, while Ca^{++} uptake into microsomes was decreased, compared to WKY. For these reasons, we have studied the activity of Ca^{++}/Mg^{++} ATPase and ATP-dependent Ca^{++} uptake in membrane fractions from brains of SHR and WKY rats. Rat brains were prepared for isolation of subcellular membrane fraction by density gradient centrifugation. Animals were sacrificed at 4 weeks of age. Ca^{++}/Mg^{++} ATPase activity were not significantly different for SHR compared to WKY. Microsomal enzyme activity was significantly reduced (25%) in SHR animals. Ca^{++}/Mg^{++} ATPase activity was significantly lower (35%) in SHR light synaptic membranes (SPM_{1-2}), while no change was seen in heavy plasma membranes (SPM_3). Also, ATP-dependent Ca^{++} uptake was significantly reduced (25%) in SHR plasma membranes (SPM_{1-2}) compared to WKY, while SPM_3 activity was not significantly different. These results demonstrate that Ca^{++} transport activity in brain nerve ending plasma membranes is reduced in SHR rats prior to the expression of measurable hypertension. No changes in systolic blood pressure were detected between the two groups. The results suggest that alterations in brain nerve ending Ca^{++} levels may genetically predispose these animals to hypertension during adult life.

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209.1 IN VIVO RELEASE OF ADRENAL CATECHOLAMINES BY FUSARIC ACID. K. Racz*, N.T. Buu, O. Kuchel*, and S. Tenneson* (SPON: S. Gauthier), Clinical Research Institute of Montreal, Montreal, Quebec H2W 1R7, Canada.

There are considerable discrepancies in estimates of catecholamine (CA) turnover based on studies utilizing different CA biosynthetic enzyme inhibitors. Since the conflicting data may be in part related to pharmacological properties of these drugs other than enzyme inhibition, we studied the effect of fusaric acid, an inhibitor of dopamine-β-hydroxylase on adrenal and peripheral CA content of the rat.

Wistar-Kyoto rats receiving fusaric acid (single intraperitoneal dose, 100 mg/kg body weight) were killed at 0, 30, 60 and 120 min after the treatment and the CA content of peripheral tissues was measured by reverse-phase high performance liquid chromatography. The fusaric acid treatment resulted in a progressive decline of adrenal epinephrine (E) and norepinephrine (NE) and an increase of adrenal dopamine (DA); the kidney and heart NE and DA contents were affected in a pattern similar to that of the adrenals. In contrast, we found marked increases of E in the kidneys and heart with a maximum response at 120 min, when kidney E increased from 7 ± 3 to 54 ± 13 pmol/kidney and heart E in both atrium and ventricle rose from 17 ± 2 to 78 ± 11 and from 38 ± 3 to 256 ± 46 pmol/organ, respectively. In addition, a large increase of E but not of NE and DA was found in the plasma of treated rats. Fusaric acid-treated bilaterally adrenalectomized rats did not exhibit any increase in plasma, heart and kidneys E concentrations.

The results suggest that fusaric acid stimulates adrenal E release in vivo, resulting in large increases of E in peripheral tissues and plasma. The decline in the adrenal E content in the adrenals is thus not only due to its decreased synthesis but also increased release. The mechanism of this potent adrenal E releasing action of fusaric acid remains unknown. (Supported by the Canadian Heart Foundation and the Medical Research Council of Canada).

209.2 ADRENAL CATECHOLAMINE RELEASE POTENTIATION BY NALOXONE DURING HEMORRHAGIC SHOCK. M. Bouvier and J. de Champlain. Centre de recherche en sciences neurologiques, Dept. de physiologie, Université de Montréal, Montréal, Canada, H3C 3T8.

Naloxone has been shown to increase mean arterial pressure (MAP) during hemorrhagic shock (HS). On the other hand the systemic administration of opiate receptor antagonist has been reported to be without effect on basal MAP. The mechanism whereby naloxone improves the blood pressure restauration in hemorrhagic hypotensive animals remains obscure. The present study was designed to evaluate the possible contribution of the sympatho-adrenal system to the pressive action of naloxone. The effects of naloxone (200 µg/kg) on MAP, heart rate (HR) and circulating catecholamines (CA) have been studied in control chloralose anesthetized rats and in rats submitted to a severe hemorrhagic hypotension. The withdrawal of blood to reach a MAP of 50 mmHg for 60 min induced a 6-fold increase in circulating epinephrine (E) but caused only a slight increase in plasma norepinephrine levels. Bilateral adrenalectomy performed 48 hours prior to evaluation abolished the E increase and greatly potentiated the plasma NE augmentation caused by HS confirming the importance of the adrenal medullary secretion during HS in intact animals. Hemorrhagic hypotension was also accompanied by a significant bradycardia which can be completely abolished by vagotomy. Naloxone given to control animals was without effects on all the parameters studied. In hemorrhagic rats however naloxone induced a significant augmentation in MAP and HR associated to an increase in plasma NE and E concentrations whereas saline injection did not significantly alter those parameters. Plasma E concentration which was already increased by the HS reached 500 ± 89 pg/ml as compared to 150 ± 71 pg/ml before naloxone administration ($p < 0.01$). The NE potentiation was smaller however and plasma levels raised from 136 ± 23 pg/ml before naloxone to 222 ± 14 pg/ml following the opiates blocker injection. The blood pressure increase and tachycardia induced by naloxone in hypotensive animals therefore appears to be related to an increased adrenal catecholamine release. This possibility is consistant with several observations suggesting that opiate peptides co-stored with CA in chromaffin cells diminish the adrenal release of CA. Whether the effect of naloxone on adrenal secretion is mediated through specific opiate receptors on chromaffin cells or through central or less specific mechanism remained to be clarified. Supported by Medical Research Council of Canada and Quebec Heart Foundation.

209.3 CATECHOLAMINE RELEASE TO HEMORRHAGE: EFFECT OF PRIOR BLOOD LOSS. D.A. Berelter, A.M. Zaid* and D.S. Gann. Brown Univ./ RI Hospital, Div.Biol.& Med.,Providence, R.I. 02902

The reflex release of adrenal catecholamines(CA) during hemorrhage(H) requires innervation, is sensitive to the rate and magnitude of H and responds similarly to repeated H provided the duration of the hypovolemic period is brief(3min). In contrast, increasing the duration of the initial hypovolemic period to 20min markedly potentiates the CA response to a subsequent H sustained 90min later. To assess the effect of prior H on H-induced CA release, adult cats were anesthetized with chloralose/urethane and sustained 2 H periods 90min apart. Catheters were placed in the aorta(MAP, HR monitor), femoral artery(H), femoral vein(blood samples) and in the brachial vein(infusions). All surgical preparation was complete by 2h prior to the initial H period. Responses to 20%H(determined by dye dilution, blood removed over 2min, returned to the animal after 20min) were compared in 3 groups of cats: a)initial period of 0%(time controls) followed 90min later by 20%H, b)initial period of 10%H followed by 20%H, and c)initial period of 20%H followed by 20% H. Blood samples were taken every 2min for the duration of each 20min experimental period and extracted plasma was assayed for CA's by HPLC with electrochemical detection. Results include:1)a marked increase in NE(>4ng/ml)during 20% H when preceded by 20%H, but only a slight response to 20%H (<1ng/ml) when preceded by 0%H or 10% H, 2)a marked increase in E(>0.9 ng/ml) to 20%H when preceded by 20%H, but only a slight response(<0.1ng/ml)to 20%H if preceded by 0%H or 10% H, 3)no significant increase in DA to 20%H in any group, 4) the total decrease and spontaneous recovery of MAP during 20%H was equal in the 3 groups and could not account for the apparent potentiation of the CA response. These data indicate that the magnitude of the initial H critically determines the CA response to a subsequent H. The potentiation of H-evoked NE and E release to 20%H is not the result of altered cardiovascular responsiveness nor of elevated CA baselines prior to the onset of the second 20%H. It is not yet known if H-evoked potentiaion of CA release by prior H is the result of altered neural input to the adrenal or the result of an increase in some circulating humoral factor. Quantitative assessment of H as a stimulus for CA release requires adequate knowledge of the magnitude(% of blood volume), rate and duration of H. In experimental designs of repeated H the prior stimulus history of the gland must also be considered. Supported by NIH Grants AM-26831 & GM-27946.

209.4 SYMPATHETIC ACTIVATION OF ADRENAL ENKEPHALIN AND CATECHOLAMINE SECRETION AFTER HEMORRHAGE IN AWAKE DOGS. W.C. Engeland*, D.F. Berelter* and D.S. Gann. Brown Univ/ RI Hospital, Div.Biol.& Med., Providence, R.I. 02902.

The opiate-like peptides, methionine and leucine enkephalin(M-Enk,L-Enk), are co-stored with adrenal catecholamines. However, the In vivo regulation of adrenal medullary secretion of enkephalins(Enk) has not been studied in awake animals. To determine if the adrenal Enk response to hemorrhage(H) varies with the magnitude of H, trained awake dogs(n=5) prepared with adrenal vein cannula 48h prior to experimentation, were bled 10 or 20% of blood volume(BV;measured by dye dilution) over 3min. Adrenal venous samples were collected prior to and for 30min after H. Plasma was extracted using Sep-Pak columns and M-Enk and L-Enk were measured by RIA. Secretory rates(M-Enk, L-Enk) were calculated as plasma concentration x adrenal plasma flow. Basal M-Enk was 600-850pg/min, with no change after 10%H. However, M-Enk increased($p<0.01$) after 20%H, with a peak of 3500pg/min at 30min. The response to 20%H was greater($p<0.01$) than to 10%H. Basal L-Enk was 350-400 pg/min; L-Enk increased($p<0.01$) after20%H, with a peak of 2000pg/min at 30min. The ratio of M-EnK to L-Enk varied from 2.5-4.0, with no change after H. To examine the relationship between M-Enk and epinephrine seretion (E), adrenal plasma E was measured using HPLC with electrochemical detection. Cross correlations(r) between M-Enk and E were computed on each of 13 experiments. In 12 of 13 there was a maximum positive correlation at a 0min delay ($p<0.01$), with r ranging from 0.46-0.97(mean,0.82). To establish if similar control mechanisms exist for adrenal Enk nd E, the effect of adrenal sympathectomy was studied. Two weeks following unilateral lumbar sympathectomy and splanchic nerve cut, awake dogs(n=5) with bilateral adrenal vein cannula were bled 20% of BV. Results showed that adrenal denervation lowered($p<0.01$) basal M-Enk and E and blocked the M-Enk and E response to H. These studies show in awake dogs that: adrenal Enk increases after moderate H; the response to H increases with magnitude of H; both M-Enk and L-Enk contribute to the response; adrenal secretion of Enk is highly correlated and synchronous with the secretion of E; and sympathetic adrenal denervation blocks the adrenal E and Enk response to H. These findings suggest that in awake animals adrenal Enk and E are regulated by the same control mechanisms. Supported by NIH GM-27946.

209.PO ACTIVATION OF THE ADRENAL MEDULLA BY CELL BODIES IN THE LOCUS COERULEUS OF THE CAT. P.J. Goadsby* (SPON: D.Rapaport). Div. of Neurol., Dept. of Med., The Prince Henry Hospital, Little Bay. 2036. Australia.

The locus coeruleus of 40 cats was electrically and chemically (l-glutamate) stimulated while both common carotid arterial resistance and catecholamine release from the adrenal medulla were measured. Stimulation lead to generalized activation of the sympathetic nervous system characterized by an immediate pressor response which was followed in the post-stimulus period by an increase of 49±10% and 44±6% in common carotid arterial resistance ipsilateral and contralateral to stimulation respectively. This later response was not affected by vagotomy or bilateral cervical sympathectomy but was blocked by high spinal cord section. The post-stimulus carotid vasoconstrictor response could be entirely eliminated by acute bilateral physiological adrenalectomy in the form of adrenal hilar clamping, an effect which was reversible if the clamps were removed. The carotid vasoconstrictor response was associated with a rise in the circulating level of noradrenaline (260%) and adrenaline (196%), which was prevented by clamping the adrenal hilum. This response was not mediated via the hypothalamus because it persisted in the decerebrate animal, nor was it merely excitation of fibres of passage since it was reproduced by microinjection of l-glutamate into the locus coeruleus. The response was blocked by phentolamine suggesting it is mediated by alpha adrenoceptors. These data represent the first conclusive demonstration that cell bodies in the brainstem are capable of activating the adrenal medulla. This fact is central to our present concept of the organization of the sympatho-adrenal axis.

209.6 ARE TYROSINE HYDROXYLASE AND PHENYLETHANOLAMINE-N-METHYL TRANSFERASE SELECTIVELY RETAINED IN DIGITONIN-TREATED ADRENAL MEDULLARY CHROMAFFIN CELLS? Katrina L. Kelner, Kyoji Morita*, Jon Rossen*, and Harvey B. Pollard. Lab of Cell Biology and Genetics, NIADDK, NIH, Bethesda, Md. 20205

Several reports have suggested that two of the enzymes involved in catecholamine biosynthesis, tyrosine hydroxylase (TH) and phenylethanolamine N-methyl transferase (PNMT), classically considered to be soluble proteins, may actually be localized on the surface of the chromaffin granule. Treatment of cultured bovine adrenal chromaffin cells with the detergent, digitonin, selectively permeabilizes the plasma membrane (Wilson and Kirschner, 1983; Dunn and Holz, 1983) and allows direct access to the cytoplasmic compartment. We have used digitonin-treated chromaffin cells to investigate the subcellular localization of TH, PNMT and dopamine β-hydroxylase (DBH), which is localized in the granules, by examining their extracellular appearance over time at two digitonin concentrations in the absence of calcium. Treatment of cultured chromaffin cells with 40 μM digitonin at 37°C for times up to 30 min caused the rapid extracellular appearance of a fraction of the cellular TH, PNMT, lactate dehydrogenase (LDH) and total soluble protein. Proteins of unknown function quantitated by SDS-PAGE and densitometry also appeared rapidly. DBH, epinephrine and norepinephrine remained in the cell, reflecting the invulnerability of the chromaffin granule to digitonin. In contrast, treatment of the cells with 10 μM digitonin at 37°C for times up to 30 min only caused the rapid extracellular appearance of a fraction of LDH, total soluble protein and proteins quantitated by SDS-PAGE and densitometry. PNMT and TH appeared in the extracellular medium at a considerably slower rate than the other proteins. As with the 40 μM treatment, DBH and the catecholamines were not released. A digitonin titration revealed that, as had been indicated by the time course experiments, only a fraction of each protein could be released from the cell by digitonin: soluble protein (40%), LDH (70%), PNMT (55%), TH (90%). In summary, TH and PNMT are selectively retained by cells treated with 10 μM digitonin, suggesting that there may be an interaction between these enzymes and a subcellular structure, possibly the chromaffin granule membrane. Additionally, digitonin-treatment allows release of only a fraction of any given protein. The nature of the digitonin-insensitive pool is unknown.

209.7 FETAL ADRENAL CORTEX STIMULATES MEDULLARY CATECHOLAMINE (CA) RELEASE-MEDIATION BY VIP. C.Y. CHEUNG, M. MALTO* AND M.A. HOLZWARTH. DIV. PERINATAL BIOL, LOMA LINDA UNIV, LOMA LINDA, CA 92350; DEPT ANAT. SCI. UNIV. ILLINOIS, URBANA, IL 61801

In the ovine fetus, circulating CA plays a major role in the control of cardiovascular dynamics. The release of CA from the adrenal medulla, the primary source of circulating CA in the ovine fetus, is subjected to regulation by the adrenal cortex. The purpose of the present study was to investigate the adrenal cortical factors involved in the stimulation of medullary CA release in the fetus. Adrenal glands from ovine fetuses of 135 days gestation were obtained, the adrenal cortex was separated from the medulla, and each tissue was separately dispersed into single cells using 0.05% collagenase. The cortical and medullary cells were independently plated in Krebs-Henseleit medium containing 0.5% BSA, and incubated at 39.5°C in a humidified atmosphere of 95% air and 5% CO_2. Following plating, the cortical cells were incubated for 2 h and the medium was collected and tested for CA stimulatory activity. To test for CA stimulatory activity, medullary cells were treated with the 2-h adrenal cortical cell medium (ACM) and the concentrations of the 3 CA's-dopamine (DA), norepinephrine (NE) and epinephrine (EPI)-released were measured at 3, 6, and 24 h using a radioenzymatic assay. Total CA release was greatly enhanced by 13, 9 and 7 folds at 3, 6 and 24 h, resp. in the presence of ACM. The stimulation of total CA was constituted by significant increases of DA, NE and EPI. Following ether extraction of ACM to remove steroids, CA stimulatory activity remained, while little CA stimulatory activity was demonstrated in the ether extract. Acidic extract of ACM contained 93% of the activity which could be destroyed by protease digestion, suggesting that the responsible factor was peptide in nature. With the recent localization of vasoactive intestinal polypeptide (VIP) in adult rat adrenal cortex, fetal cortex was studied for the presence of VIP using PAP immunocytochemical method. VIP-immunoreactivity was observed in abundance in fine varicose fibers which ran among groups of glomerulosa cells and radially among fasciculata-reticularis cells. The immunoreative fibers were distributed throughout the entire thickness of the cortex. In addition, VIP significantly stimulated total CA release from medullary cells by 100% and 126% at 6 and 24 h, resp. The results suggest that fetal adrenal cortex contains high concentrations of VIP which can enhance CA release from the medulla. Thus, fetal adrenal cortex may exert major influence on medullary CA secretion through the release of VIP.

209.8 ANALYSIS OF CATECHOLAMINE BIOSYNTHESIS IN CULTURED ADRENAL MEDULLARY CHROMAFFIN CELLS BY HPLC: INFLUENCES OF SECRETAGOGUES ON BIOSYNTHETIC RATE AND RELEASE. Robert A. Levine, Katrina L. Kelner, and Harvey B. Pollard. Lab of Cell Biology and Genetics, NIADDK, NIH, Bethesda, Md. 20205

Cultured adrenal chromaffin cells provide an excellent system for investigating regulation of catecholamine biosynthesis and release. To this end, we have developed an HPLC reverse-phase system to separate tyrosine, the catecholamines (dopa, dopamine, norepinephrine, epinephrine) and their major metabolites (DOPAC, HVA, normetanephrine, VMA, metanephrine, and DOPEG). Fluorescence detection (Perkin-Elmer LS-4) for unlabelled compounds minimized baseline variations and allowed the use of gradient elution. Mobile phase (10 mM phosphate, 10 mM TCA, 0.02% SDS) containing 5% acetonitrile was run for 15 min followed by a 15 min gradient to 20% acetonitrile. An in-line radioactivity detector (Radiomatic H-S Flo-One) after the fluorometer was used to monitor the incorporation of radioactivity into each intermediate from radiolabelled tyrosine.

Incubation of cultured chromaffin cells with 20 μM ^{14}C-tyrosine for various times up to 2 h established a basal synthetic rate for each intermediate. Treatment of the cells with a battery of secretogogues, including nicotine, high potassium, veratridine and oubain caused increases in the biosynthetic rates of the catecholamines that varied in magnitude among the compounds tested. In addition, the tested secretagogues varied in the specificity of their released products, suggesting differences in their mechanisms of release. In summary, we have developed an HPLC system for separation and quantitation of catecholamines with which we can analyze the complete catecholamine biosynthetic pathway in adrenal chromaffin cells. This system has been used to analyze the effects of various secretagogues on biosynthetic rates and released catecholamines.

209.9 POTASSIUM AND NICOTINE STIMULATED CATECHOLAMINE RELEASE FROM CULTURED CHROMAFFIN CELLS ARE MEDIATED BY TWO DIFFERENT MODES OF CALCIUM FLUX. E. Heldman*, M.A. Levine* K. Morita*, H.B. Pollard, Laboratory of Cell Biology and Genetics, NIADDK, N.I.H., Bethesda, Md. 20205

In adrenal medulla the rate of catecholamine release decreases with continuous stimulation. We explored this phenomenon using cultured bovine chromaffin cells stimulated with several secretagogues. As expected, the rate of K^+ (50 mM) and nicotine (62 µM)-stimulated catecholamine secretion rapidly decreased during continuous stimulation. In contrast, veratridine (20 µM)-induced release was prolonged and resulted in greater depletion of cell catecholamines. Thus, under nicotine or K^+ stimulation, the rapid decrease in catecholamine release was not due simply to depletion of a releasable pool. We then examined catecholamine release and Ca^{+2} entry during sequential stimulations. During the second step of a repetitive K^+ stimulation, the amount of catecholamine released was dramatically decreased. However, when extracellular Ca^{+2} was omitted or $LaCl_3$ (2 mM) added to the medium in the first step stimulation, catecholamine release was not decreased during the second step. These data indicate that Ca^{+2} entry must occur to produce decreased release in a subsequent K^+ stimulation. In contrast to K^+ stimulation, omission of extracellular Ca^{+2} during a first step of nicotine stimulation still resulted in decreased release during a second step with nicotine, indicating that desensitization of the cholinergic receptor was probably responsible for the decreased release. When $^{45}Ca^{+2}$ influx was measured during a single K^+ stimulation, we observed a rapid inactivation of Ca^{+2} entry with a rate that was nearly identical to that of decreasing release. Ca^{+2} influx during nicotine stimulation did not inactivate a subsequent K^+ induced release and only partially inactivated K^+ induced Ca^{+2} influx. These data indicate that the inactivation of Ca^{+2} channels requires Ca^{+2} entry via the subsequently inactivated channels and that nicotine induces Ca^{+2} entry via a different route than K^+. The existence of at least two sites of Ca^{+2} entry was also suggested by experiments with hyperosomotic media. We found that elevated osmotic strength depressed K^+ induced Ca^{+2} influx but not nicotine induced Ca^{+2} influx. We conclude that the two independent Ca^{+2} channels are inactivated by separate mechanisms. One population of channels, is activated by voltage changes, and inactivated by Ca^{+2} itself. The other population is activated by nicotinic agonists and indirectly inactivated by desensitization of the receptor.

209.10 TRANSFORMATION OF CHROMAFFIN CELL TO PC12 CELLS INVOLVES SIMULTANEOUS PHENOTYPIC CHANGES IN MONOAMINE OXIDASE ACTIVITY AND TYRAMINE RELEASABLE POOLS OF NOREPINEPHRINE. M.B.H. Youdim*, H.B. Pollard and E. Heldman*. (Spon: N. Bernick). Lab. of Cell Biology and Genetics, NIADDK, NIH, Bethesda, Md. 20205

Monoamine oxidase (MAO) inhibitors have little effect on the metabolism of nonepinephrine (NE) in the bovine adrenal gland in spite of the substantial MAO activity present in the medulla. Adrenal medulla MAO activity have been differentiated into both A and B forms. The A form preferentially deaminates NE and serotonin while the B form deaminates non-hydroxylated amines (e.g. phenylethylamine). Dopamine and tyramine are substrates for both enzyme forms. The isolated chromaffin cells (CC) are able to metabolize monoamines by a process of uptake, deamination, and storage. Its MAO activity, however, is entirely type B (Youdim et al. Science, 1984 in press), thus explaining the lack of NE metabolism in these cells. The presence of MAO-B in CC with poor affinity for NE and the lack of effect of MAO inhibitors on its NE content, are compatible with the physiology of the cell to synthesize and store large amount of catecholamines. It is paradoxical that while sympathetic neurons and adrenal medullary chromaffin cells originate from the neural crest, their MAO activities appears to be distinctly different, since nerve endings contain primarily MAO-A. As a possible window into the relationships between these two cell types, adrenal-chromaffin cell-derived PC12 has been observed to have properties shared by both. PC12 cells have a close resemblance to NE nerve endings, because MAO inhibitors do elevate its cytoplasmic catecholamine levels. Therefore, we examined MAO activity in PC12 cells and found that it was exclusively of the A type, very much like that of the nerve endings. This may indicate nerve ending-like properties of PC12 cells, in as much as PC12 cells loaded over a 15 min period with ^{3}H-NE released up to 8% of total labelled material when stimulated with 1 mM tyramine. By comparison 50 mM KCl released twice as much. The additive effects of tyramine and KCl induced release would indicate the existance of two separate pools for NE, presumably a cytosolic and secretory granule. In contrast, CC released virtually no ^{3}H-NE even upto 1 mM tyramine, in spite of a substantial KCl (50 mM) induced release. We therefore suggest that since PC12 is derived from CC the genes that control MAO-type and tyramine-susceptibility may be linked and their coincident expression related to the transformation of the CC.

209.11 ASCORBIC ACID REGULATION OF NOREPINEPHRINE BIOSYNTHESIS IN ISOLATED CHROMAFFIN GRANULES FROM BOVINE ADRENAL MEDULLA. K. Morita*, M. Levine*, and H. Pollard (Spon: D. Bergstrom). Lab of Cell Biology and Genetics, NIADDK, NIH, Bethesda, Md. 20205

Ascorbic acid is the optimal electron donor for isolated dopamine beta hydroxylase, but the importance of this observation to dopamine beta hydroxylase function in chromaffin granules has been open to question. To investigate this problem, we studied the influence of ascorbic acid on synthesis of norepinephrine from dopamine in isolated bovine chromaffin granules. Chromaffin granules incubated with (^{3}H) dopamine and Mg-ATP were able to transport the catecholamine and to synthesize (^{3}H) norepinephrine. With the addition of ascorbic acid, chromaffin granules doubled the amount of (^{3}H) norepinephrine biosynthesis as compared to control. (^{3}H) dopamine found in the same granules in the presence of ascorbic acid was slightly less than control, while total (^{3}H) catecholamine was identical under both conditions. Since dopamine beta hydroxylase is located within granules, we then investigated ascorbic acid uptake. Ascorbic acid was found to not be transported at all into chromaffin granules, in the presence of Mg-ATP and in the presence or absence of (^{3}H) dopamine, and as measured both by (^{14}C) ascorbic acid uptake or directly by HPLC. Despite lack of ascorbic acid uptake, ascorbic acid increased norepinephrine biosynthesis in chromaffin granules under many conditions. Enhancement occurred at all concentrations of Mg-ATP between 0.25 mM and 5.0 mM, although the optimal Mg-ATP concentration appeared to be 2.5 mM. Likewise, ascorbic acid doubled new (^{3}H) norepinephrine biosynthesis whether external (^{3}H) dopamine concentration was 20 µM, 50 µM, or 100 µM. The ascorbic acid effect was evident at concentrations as low as 200 µM external ascorbate, and the optimal concentration was 2 mM, a concentration nearly identical to that previously found by us in chromaffin cells. Ascorbic acid enhancement of norepinephrine biosynthesis could not be duplicated by other reducing agents such as NADH, glutathione, homocysteine, or thiourea. We conclude that ascorbic acid specifically enhances norepinephrine biosynthesis from dopamine in isolated chromaffin granules, without uptake of ascorbic acid Our results strongly suggest the existence of an electron transport system across the chromaffin granule membrane

209.12 SEROTONIN UPTAKE BY ADRENAL MEDULLARY CELLS. M.Holzwarth and C.Sawetawan*. Dept. Anatomical Sciences, Univ. of IL, Urbana, IL 61801.

Serotonin-like immunoreactivity has been previously identified in medullary cells of the rat adrenal gland using immunocytochemistry. Double labelling immunocytochemical experiments lead to the conclusion that serotonin and epinephrine are found in the same cells. The immunostaining of these cells is modifiable with various pharmacological agents known to affect serotonin (5-HT) content of serotonergic neurons: p-chlorophenylalanine (synthesis inhibitor) and reserpine (monoamine depleter) treatment reduced 5-HT immunostaining, L-tryptophan (precursor) and pargyline (MAO-inhibitor) augmented immunostaining and p-chloroamphetamine (5-HT releaser) decreased immunostaining.

In order to further evaluate the physiological significance of adrenal medullary 5HT-immunoreactivity, the uptake characteristics of 5-HT and its precursors, 5-hydroxytryptophan and L-tryptophan, have been investigated. 5-HT uptake was first demonstrated with immunocytochemistry of adrenals which had been previously depleted of 5-HT stores with reserpine; when incubated with $1x10^{-5}$ M 5-HT, augmented immunostaining was observed. Uptake was further investigated by incubation of quartered adrenals in Krebs-bicarbonate buffer containing $1.2x10^{-7}$ M ^{3}H-5-HT at 37 C. The amount of radioactive amine taken up was determined by scintillation counting of solubilized adrenals or of perchloric acid extracts of homogenates. Adrenals continued to take up 5-HT for at least 60 min with the greatest rate of uptake occurring during the first 15 min. Reserpine pretreatment had no significant effect on the amount or rate of uptake. The uptake was verified by measuring ^{3}H-5-HT uptake in the presence of increasing concentrations of unlabelled 5-HT (10^{-8} to 10^{-4} M). To ascertain non-specific uptake adrenals were incubated at 4 C; uptake was reduced by at least 80%. Specificity of uptake was determined by isotope dilution experiments and by addition of $1x10^{-5}$ M fluoxitine (uptake inhibitor) which reduced uptake to 33% of controls. Cellular localization of ^{3}H-5-HT has been verified with autoradiography. Significant uptake of ^{3}H-5-hydroxytryptophan has also been demonstrated. These results provide evidence for the presence of a specific serotonin uptake mechanism in the adrenal medulla of the rat.

209.13 INCORPORATION OF ^{35}S AND ^{32}P FROM LABELLED NUCLEOTIDES INTO SAPONIN-SKINNED CHROMAFFIN CELLS. J.C. Brooks and M. Brooks*. Marquette University School of Dentistry, Milwaukee, WI 53233.

We have previously demonstrated a calcium enhanced incorporation of ^{35}S from the nucleotide ATP S into saponin-skinned, cultured chromaffin cells. The rationale for use of this nucleotide was that cellular kinases can use it for thiophosphorylation of proteins while the resultant thiophosphoproteins are resistant to dethiophosphorylation by cellular phosphatases. Thus it should be possible to lock cellular phosphorylation-dependent reactions in the thiophosphorylated state and determine their relationship to the secretory process. When skinned cells were incubated for 5-30 min with tracer concentrations of [^{35}S]ATPγS, the label appeared primarily in two proteins, designated as Protein-I (MW= 54,000 daltons) and Protein-II (MW= 43,000 daltons). Protein-I was more heavily thiophosphorylated in control cells and Protein-II was more heavily thiophosphorylated in the calcium stimulated cells. Neither protein was thiophosphorylated in intact cells; a series of other, higher molecular weight proteins incorporated label in stimulated intact cells.

In parallel experiments with [^{32}P]ATγP, label was incorporated into Proteins-I and II of skinned cells as well as a number of other proteins. However, the major difference was a much lower total incorporation into the stimulated cells compared to the unstimulated controls. This was not due to protein loss from the stimulated cells, which actually contained significantly more protein than unstimulated cells at the end of the usual 30 min. incubation period.

Thiophosphorylation causes both the abolition of secretion in skinned cells and the incorporation of thiophosphate into specific cellular proteins. In contrast, several other proteins were predominantely phosphorylated when ATP was used as the substrate for secretion. Thus Proteins-I and II appear to have a direct role in the secretory process, while several others do not. Since the labelled nucleotides were present in tracer quantities, the greater phosphorylation of control cells than stimulated cells probably reflects the rate of turnover of labelled phosphate from phosphoproteins.

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209.14 CHARACTERIZATION OF ADRENAL MEDULLA HOMOGENATES. S.W. Carmichael, G.L. Pfeiffer,* D.R. Studelska* and W.S. Brimijoin. Section of Anatomy and Department of Pharmacology. Mayo Clinic, Rochester, MN 55905.

This study is the first phase of our attempt to isolate mitochondria-chromaffin vesicle complexes that have been identified in vivo (Carmichael and Smith, Cell Tiss. Res. 191:421-432, 1978). We have characterized homogenates of the adrenal medulla by biochemical and morphological criteria and will later report on a fraction enriched in mitochondria-chromaffin vesicle complexes. Fresh bovine adrenal medullas were gently homogenized in a TCS Continuous Tissue Homogenizer. The supernatant from a 800xg 10 min centrifugation was spun at 15,000xg for 15 min. This pellet was resuspended in buffered sucrose and mixed into a solution of 60% Percoll and 0.28M sucrose. The mixture was spun at 42,000xg for 80 min, allowing for formation of the Percoll gradient and separation of organelles based on buoyant density. Fractions were analyzed for monoamine oxidase (MAO, marker of the outer mitochondrial membranes), dopamine ß-hydroxylase (DßH) and catecholamines (markers for chromaffin vesicles), and protein. The same fractions were also examined by routine transmission electron microscopy (TEM), negative stain TEM of smears, and scanning electron microscopy (SEM) of filtrates. Based on particle size, mitochondria and chromaffin vesicles could be identified by negative stain and SEM. Also, Percoll particles (20nm) could be seen by negative stain. DßH peaks were found in the lightest fractions and presumed to be soluble contents of lysed vesicles. Intact vesicles were in a fraction heavier than mitochondria as shown by their association with epinephrine and norepinephrine. The fractions were assayed for membrane-bound DßH after subjecting the vesicles to freeze-thawing and sonication in a hypotonic buffer, high-speed centrifugation, and resuspension in a Triton-containing buffer. This DßH peak correlated with the catecholamine peak. Mitochondria were in a fraction containing the highest protein concentration. The MAO activity of both the medulla and the cortex appeared to be both Type A and B, as evidenced by studies with selective inhibitors. The examination of fractions by TEM confirmed the findings with markers. Mitochondria-chromaffin vesicle complexes could be identified in fractions at the MAO-DßH interface. This work was supported by NIMH37937 and Mayo Foundation.

NEURAL CONTROL OF IMMUNE SYSTEM

210.1 NODOSE AND SUPERIOR CERVICAL GANGLIA PROJECTIONS TO THE RAT THYMUS GLAND. K.Bulloch, E.Roth* and M.R. Cullen.* (SPON: T. Melnechuk) Dept. of Neuro., Div. of Neuroimmunol. Sch. of Med. S.U.N.Y. Stony Brook, N.Y. 11794.

The thymus is innervated by fibers of the ANS.Horseradish peroxidase (HRP) histochemical studies revealed that part of the source of this innervation is derived from the brainstem's n. ambiguus, n. retrofacial and from three discrete cell columns in the ventral horn of the cervical spinal cord. To further characterize thymic innervation, an HRP study was undertaken to determine if the superior cervical and nodose ganglia projected to the rat thymus. HRP (5ul) was injected via a Hamilton microliter syringe into both thymic lobes of six-week-old Sprague Dawley rats. The rats were perfused 24 hours post-injection and the nodose and superior cervical ganglia were removed, frozen and cut serially 36 um thick. The tissue was processed for TMB retrograde transport reaction. Control rats received HRP injections into the pectoral muscles ventral to the thymus. Injections of HRP into the left and right thymic lobes produced labeling of neurons in the ipsilateral nodose and superior cervical ganglia. In the nodose ganglia large neurons (40 um) were labeled with HRP in the area immediately adjacent to where the superior laryngeal nerve joins the ganglia. In the superior cervical ganglia labeled neurons were observed within two areas. One group of small labeled neurons (24 um) was evident in the caudal pole of the ganglia, whereas a group of medium size neurons (32 um) was labeled in the mid-region of the ganglia. Control HRP injections yielded no labeled cells in either the nodose or superior cervical ganglia. The mammalian nodose ganglia are sensory ganglia whose axons run within the vagus nerve to provide sensory information from visceral receptors to special centers within the brain. The presence of such nerves within the thymus clearly demonstrates the ability of this primary immune system organ to directly communicate via neuroanatomical pathways with discrete regions of the central nervous system. The superior cervical ganglia are the primary ganglia of the sympathetic chain and are thought to provide vasoglandular sympathetic innervation to tissues of the head or neck e.g. pineal. Taken collectively these findings add valuable new information about the types of innervation that participate in thymic innervation and furthers our basic understanding of the complex pathways of neuroimmune integration.

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210.2 PRIOR EXPOSURE OF RATS TO ESCAPABLE SHOCKS REDUCES THE IMMUNOSUPPRESSIVE EFFECTS OF INESCAPABLE SHOCKS. J. E. Kelsey and A. C. McGrath.* Dept. Psychology, Bates College, Lewiston, ME 04240.

Recent research has demonstrated that exposure to uncontrollable, but not controllable, stressors can enhance susceptibility to diseases, including cancer, by suppressing the function of the immune system. Based on findings that prior exposure to controllable stressors can reduce some of the behavioral effects of uncontrollable stressors (e.g., learned helplessness), the intent of this study was to determine if prior exposure to escapable shocks in rats would reduce the immunosuppressive effects of subsequent inescapable shocks.

During the initial session, rats were placed in groups of three in wheel-turn chambers. Rats in the escapable shock group were allowed to escape 80 tail shocks (1.0 mA), delivered on a VT 60-sec schedule, by turning a wheel at the front of the chamber. Rats in the inescapable shock group were yoked to rats in the escapable shock group and received tail shocks whenever the latter group did. Wheel-turning by rats in the inescapable shock group had no effect on the amount of shock received. Rats in the third group served as unshocked controls. Two days later, the rats in the escapable and inescapable shock groups received 80 5-sec inescapable tail-shocks delivered on a VT 60-sec schedule in the same chambers. Rats in the control group were again unshocked. One day later, all rats were exposed to five 5-sec "reminder" footshocks (0.8 mA), and the function of their immune system was then assessed by measuring the proliferation of splenic lymphocytes in response to the T-cell mitogen concanavalin A (Con A).

As expected, exposure to two session of inescapable shocks caused a significant reduction in mitogen-stimulated lymphocyte proliferation. More important, prior exposure to escapable shocks completely eliminated the immunosuppressive effects of the inescapable shocks. Furthermore, this "immunizing" effect of prior escapable shocks was evident even when the escapable shocks occurred 21 days prior to the inescapable shocks and even when the rats were subsequently exposed to two sessions of inescapable shocks. These data suggest that prior exposure to controllable stressors are capable of reducing or eliminating physiological as well as behavioral effects normally produced by uncontrollable stressors, and indicate that behavioral "immunization" may help maintain physical as well as mental health.

210.3 EFFECTS OF STRESS ON IMMUNE FUNCTION IN MICE AND RATS. E. Gamzu, G. Vincent, N. Tare, W. Benjamin, J. Farrar, and A.C. Sullivan, Hoffmann-La Roche Inc., Nutley, NJ 07110

We have attempted to establish a model of stress-induced immune suppression (SISS) in order to study possible drug effects in psychophysiological disorders. The stressors have ranged from physiological (e.g. restraint/shock) to primarily psychological ("learned helplessness"; LH). Adult male mice were injected with sheep red blood cells and immediately restrained in a supine position (Vincent et al., 1984) in a cold (7-9°C) environment for 3 hr, or exposed to 60 inescapable shocks (1.6 mA, 6 sec). The mice were sacrificed 4 days later and the number of plaque forming cells (PFC) per 10^6 spleen cells were counted as a measure of immune function (IF). IF was significantly reduced in restrained mice ($\bar{x}$=180±57) compared to controls ($\bar{x}$=1953±591), but not in the shocked mice. In a second experiment, mice exposed to 4 daily sessions of 60 shocks from the day of immunization until sacrifice had lower PFC counts ($\bar{x}$=407±478) than control mice ($\bar{x}$=1183±293). When mice were exposed to 3 hr of supine restraint at room temperature, we were able to demonstrate decreased mitogen responses to PHA, ConA, LPS, and PWM as well as reduced PFC (pooled data $\bar{x}$=380 vs 1980) and a significant decrease in thymic weight ($\bar{x}$=26.9 vs 47.1 gm). While these effects are large, we have had considerable difficulty in consistently reproducing these results despite attempts to control for strain, temperature, housing, etc.

Rats given access to a running wheel and fed 1 hr each day develop multiple indices of stress within 4-7 days (activity stress (AS), Pare and Vincent, 1982), including a possible SIIS. Exposure of adult male rats to AS for 6 days significantly reduced thymic weights ($\bar{x}$=106.4 vs 276.1 gm) and decreased the mitogenic response of spleen cells to LPS, PWM, PHA, and ConA. The third stressor was LH (Laudenslager et al., 1983) in which adult male rats were exposed to escapable or an identical pattern and amount of inescapable shock (64 shocks, 1.0 to 1.6 mA). The psychological stress of lack of control decreased thymic weight of the inescapable group ($\bar{x}$=376.2 vs 443.4 gm) and its mitogen response to LPS and PWM (but not to ConA or PHA). We are currently assessing the reliability of the LH effect. The immune system is clearly sensitive to a variety of stressors, but the response is variable; one possible explanation is that the SIIS might be an indirect rather than a direct effect of stress.

210.4 EARLY SOCIAL SEPARATION EXPERIENCES IN INFANT MONKEYS AND ADULT IMMUNITY.

M.L. Laudenslager, J. Capitanio*, M.L. Reite, and R. Harbeck*, University of Denver and University of Colorado School of Medicine, Denver, CO 80208.

Brief separations of infant monkeys from their mothers have been shown to be accompanied by disruptions of behavioral, physiological, and immunological function. We have recently noted long-term behavioral alterations in adult monkeys briefly separated from their mothers as infants with regard to their social interactions with other monkeys.

In the present study, we examined several aspects of immunological functioning in nine adult pigtail (M. nemestrina) monkeys, four of whom experienced a 10-14 day maternal and/or peer separation during the first year of life. On the same day (at ages of 4.4 to 7.7 years) blood samples were obtained from all animals, and several aspects of immunity were studied, including measurement of total white blood cell counts, differential white cell counts, levels of specific immunoglobulins (IgA, IgG, and IgM), rheumatoid factor, and the lymphocyte proliferative response to mitogens (PHA, ConA, and pokeweed). At the same time on different days for each monkey, each monkey was rapidly removed from its social group, and a blood sample was obtained for cortisol determination.

For nonstressed animals, we found no differences between groups with regard to basal cortisol levels, immunoglobulin levels, rheumatoid factor, total white cell counts, or differential white cell counts. However, for all three mitogens tested, the previously separated monkeys showed statistically significant ($p<.05$) lower lymphocyte proliferative responses than nonseparated controls. These findings suggest that another effect of early social separation in monkeys is an alteration in the ability of B- and T-cell lymphocytes to undergo mitogenic transformation. (Supported by USPHS MH 37373 and MH 19514).

210.5 NK CELL ACTIVITY, HYPERCORTISOLEMIA AND DEPRESSION. H.A. Nasrallah, Z.K. Ballas*, S. Chapman*. V.A. Medical Center and Depts. of Psychiatry and Medicine, University of Iowa College of Medicine, Iowa City, IA.

Corticosteriods have been reported to exert an inhibitory effect on certain immune responses, such as lymphocyte number and function. We have previously shown that depressed patients with hypercortisolemia (indicated by dexamethasone nonsuppression of cortisol) have significantly lower lymphocyte number compared to depressed patients with normal dexamethasone suppression. To examine the effects of hypercortisolemia on lymphocyte function we studied the level of Natural Killer (NK) cell activity in depressed patients with and without dexamethasone nonsuppression; NK cells are believed to be involved in tumor surveillance.

62 male patients admitted to an acute psychiatric ward with major depression (N=41) mania (N=10) and alcoholism (N=11) consented to participate in the study. Exclusion criteria included major medical illness, intake of steroids, analgesics and antiseizure drugs. Dexamethasone testing was done after collection of blood for the study. Peripheral blood lymphocytes were obtained by Ficoll-Hypaque sedimentation and were examined for the NK cell activity. We used a standard 4-hour ^{51}chromium release assay to measure the lytic activity of the NK cells. The target cells employed were K562 tumor cells.

There were no significant differences in NK cell activity among the depressed, manic or alcoholic/depressed groups. No differences emerged between dexamethasone suppressors and nonsuppressors in the entire sample and within each group.

The data suggest that NK cell activity is not significantly altered in depression, mania or alcoholism with secondary depression, regardless of dexamethasone status. The implications of these results for immune function in affective illness are discussed.

210.6 GLUCOCORTICOID AND IMMUNE FUNCTION IN DEPRESSION: RELATION TO THE DEXAMETHASONE SUPPRESSION TEST. G.J. Gormley*, M.T. Lowy, A.T. Reder*, V.D. Hospelhorn*, J.P. Antel*, and H.Y. Meltzer. Dept. of Psychiatry, Neurology and Ben May Laboratory, Univ. Chicago Pritzker Sch. Med., Chicago, IL 60637

Several lines of evidence suggest that glucocorticoid resistance is present in some patients with major depression. We have developed a protocol utilizing neuroendocrine, biochemical and immunological techniques to assess glucocorticoid receptor binding and function in lymphocytes of depressed patients and normal controls following an in vivo dexamethasone (DEX) challenge. Serum cortisol levels were measured by RIA, glucocorticoid receptor levels by Controlled Pore Glass (CPG) bead assay, and mitogen-induced lymphocyte proliferation by ^{3}H-thymidine uptake during a standardized dexamethasone suppression test (DST). Our results indicate that suppression of serum cortisol ($<$ 5 ug/dl) following 1 mg of oral DEX in both depressed and control subjects (N=10) was associated with a decrease both in the lymphoproliferative response to mitogen stimulation and in the numbers of cytoplasmic receptors in circulating lymphocytes (20% and 39% reduction respectively). In contrast, failure to suppress cortisol in depressed and control subjects (non-suppressors, N=6) was associated with no change in the mitogen responses or receptor levels. Thus, these results suggest that DST nonsuppression is related to an abnormality in glucocorticoid receptor function. We further observed that the degree to which post-DEX serum cortisol levels were suppressed correlated with receptor changes ($r=0.63$, $p < 0.01$) and with response to mitogens such as phytohemagglutinin (PHA: $r=0.65$, $p < 0.001$) and concanavalin A (Con A: $r=0.54$, $p < 0.01$). A comparison between the DEX-induced receptor and mitogen changes from the same subjects showed highly significant correlations (PHA: $r=0.79$, $p < 0.001$, and Con A: $r=0.59$, $p < 0.05$). These results demonstrate that suppressors and nonsuppressors differ at the molecular, cellular and hormonal level and that these changes occur in parallel, representing a continuum of sensitivities to DEX rather than a dichotomous response. Lymphocytes appear to be a useful peripheral tissue to study glucocorticoid receptors and function in affective disorders.

210.7 BONE MARROW TRANSPLANT FROM AGED TO YOUNG MICE PRODUCES BRAIN REACTIVE ANTIBODIES AND CONCURRENT ACCELERATION OF LEARNING/MEMORY DEFICITS. K. Nandy*, H. Lal, M. Bennett*, and D. Bennett. V. A. Hospital, Bedford, MA 01730, Boston Univ. School of Medicine, Boston, MA 02118, and Dept. of Pharmacology, Texas Coll. of Osteopathic Medicine, Fort Worth, TX 76107.

Rate of acquisition of active avoidance responses in New Zealand Black (NZB) and C57BL/6J mice is inversely related to the presence, in serum, of brain reactive antibodies (BRA) selective for neuronal antigens. Deficits in learning and BRA exhibit age-related increases in C57 mice, whereas NZB mice show higher serum BRA titers and slower learning when much younger (Nandy, et al. Life Sci. 33: 1499, 1983; Spencer et al., Neuroscience Abs. 9: 96, 1983). In order to test for a cause and effect relationship between BRA and the learning deficit, and to further investigate mechanisms and sites of these abnormalities, bone marrow and spleen cell suspensions were transferred from aged into young C57 mice (e.g., AGED-YOUNG) along with other groups of transfers for appropriate controls. Prior to the transfer, the recipient mice were irradiated to inactivate their own immune system and, after 3-5 months, the recipient mice were investigated for simultaneous occurrence of BRA serum titers and deficits in learning of a one-way conditioned avoidance response. Comparison to like-aged, non-recipient controls showed no effect of AGED-AGED or YOUNG-YOUNG transfers on either BRA or learning. These measures varied only as a function of age, with all the aged mice showing higher BRA and slower learning. On the other hand, young recipients of immunity complement from aged donors showed high BRA titers and an accelerated learning deficit. In all high BRA groups, including NORMAL-AGED, AGED-AGED, YOUNG-AGED, and AGED-YOUNG groups, the number of trials required to learn the active avoidance response was increased two-fold from that for low BRA groups. The simultaneous increase of BRA and learning deficits in the young mice resulting from transfer of immunopoietic apparatus from the aged mice suggests that the learning deficits could be caused through an immunological mechanism. Furthermore, behavioral deficits associated with the ageing process may involve autoimmune mechanisms as well. Supported by Research Funds of Veteran Administration, US Public Health Service Grant NS-129624 and National Institute of Aging Grant 1 RO 3 AG03623.

210.8 DETECTION OF IMMUNO-REACTIVE THYMOPOIETIN IN MOUSE CENTRAL NERVOUS SYSTEM. J.S. Schweitzer*, R.H. Brown*, T. Audhya*, G. Goldstein*, M.A. Dichter. (Spon: K. Harris). Neurology Service, Massachusetts General Hospital, Boston, MA 02114, Department of Neuroscience, Children's Hospital, Boston, MA 02115; Ortho Pharmaceutical Company, Raritan, NJ 08869.

The thymic polypeptide thyompoietin (TP) was initially identified by its ability to impair neuromuscular conduction and subsequently found to induce T and B cell differentiation. We have investigated the possibility that TP or a related peptide may exist in brain and spinal cord. With a radioimmunoassay a thymopoietin - immunoreactive substance (TP-IRS) was detected in homogenates (10% weight/volume in phosphate buffered saline) of mouse brain and spinal cord as early as the thirteenth embryonic day at levels of 10 - 20 ng/ml. Levels were higher in spinal cord, peaking at birth (40 ng/ml in cord, 18 ng/ml in brain) and falling in subsequent weeks. By RIA, TP-IRS was also detected in supernatants from mouse neuroblastoma (NIE-115) and primary spinal cord cultures; supernatants of human astrocytic and meningeal tumors and and mouse primary astrocyte cultures did not contain detectable TP-IRS. Using affinity-purified rabbit anti-thymic TP globulin, immunofluoresent staining was seen in mouse spinal cord cultures in association with nuclear membranes of neurons and large, flat background cells. Staining was abolished by prior incubation of anti-TP globulin with purified TP. From supernatants of NIE-115 cells grown with ^{3}H-leucine and ^{3}H-lysine radiolabelled proteins of approximately 8,000 and 4,500 daltons were isolated by affinity chromatography with an anti-TP column. When injected into mice, these proteins partially blocked neuromuscular conduction as assessed by a decrement in the compound muscle action potential following repetitive stimulation. The dose-response of this effect was comparable to that of the active fragment of TP, TP-5 pentapeptide. It remains unclear whether the TP-IRS identified in this study is related to thymic TP or simply a cross-reactive substance although the comparable neuromuscular effects suggest the former interpretation. The function of TP-IRS remains to be defined.

210.9 ACETYLCHOLINESTERASE (AChE), THE THYMUS GLAND AND THE EFFECTS OF AN ANTI-AChE INHIBITOR ON A T-CELL DEPENDENT HUMORALIMMUNE RESPONSE. S.Bossone*, S.Cohen*, A.Ho*, B.Stricker*,M.Philips*, M.R.Cullen* and K. Bulloch. Dept. of Neuro.,Div. Neuroimmuno., Sch. of Med. S.U.N.Y. Stony Brook, N.Y. 11794.

AChE identified within nerves of the mouse thymus from early embryonic development through adult life can be used as an index of cholinergic innervation. Agents that supress antigen specific, t-cell dependent immune responses have been shown to increase the intrathymic AChE activity and decrease cholinergic innervation within the thymus. We have sought to characterize the predominant species of AChE within the thymus. We have also potentiated intrathymic cholinergic innervation and examined the antigen specific t-cell dependent humoral immune response. Crude extracts of AChE were prepared, and their kinetic properties found The Km and Vmax were found to be 6.3×10^{-4}M and 1.3×10^{-5}uM of Acetylcholine hydrolyzed per min., respectively. The specific activity of this enzyme was not affected by the doses in the range of 10^{-4} to 10^{-5} M of the non specific esterase inhibitor iso-OMPA but were inhibited by 10^{-5} to 10^{-6}M of the specific inhibitor BW284c51; substantiating the presence of true AChE enzyme. Preliminary sucrose gradient purifications indicate that the 16S and 3S forms are the predominant species of the thymic enzyme. The LD 50 for the specific AChE inhibitor BW284c51 and the optimal dose for its use in the immunological assay were evaluated in BALB/C mice. A dose which produced 40% inhibition of the thymic AChE was injected i.p. into mice 24 hours after the inoculation of the antigen DNP-OVA. Control mice received saline injections. Ten days later retro-orbital blood was tested for the Ig G_1 anti-DNP humoral immune response using an ELIZA assay. Test mice showed a six-fold increase in Ig G1 response to the t-cell dependent antigen, DNP. These results are similar to those produced in other species by less specific inhibitors of AChE, and are the reciprocal of studies which show that reagents which increase thymic AChE levels reduce the t-cell dependent immune response to DNP-OVA. This study does not show at what level these cholinergic agents exert their in vivo effect, i.e., within the thymus other immune organs or within the central nervous system pathways involved with the modulation of immunity. Furthermore, although AChE is considered nerve-related it is possible that the enzyme may have another role within the thymus, unrelated to nervous system regulation.
(supported by NIH Grant #NS18401)

210.10 DISTRIBUTION OF CHOLINERGIC RECEPTORS IN THE THYMUS DURING THE DEVELOPMENT OF NORMAL AND STAGGERER MUTANT MICE. A. Rossi* and E. Trenkner. Dept. of Pharmacology, N.Y.U. Medical Center, New York, NY 10016.

The control of the immune system development and its functions has been tentatively linked to neuronal innervation (1). If the innervation plays a role in the development of lymphoid tissue abnormal innervation and/or delayed expression of neurotransmitter receptors might cause abnormal development and subsequently immune deficiency.

We have shown that the neurological mutation staggerer (sg/sg) not only affects the development of the cerebellum but also causes developmental and regulatory changes of the immune system (Trenkner and Hoffmann, these Abstracts). Bullock and Loy (2) have reported that the innervation pattern into sg/sg thymus is abnormal. Very little is known, however, about the expression of neurotransmitter receptors during thymus development.

This study was performed to elucidate the expression of acetycholine receptors during the development of normal and sg/sg thymus. We have observed: 1. that the number of specific binding sites for α-bungerotoxin increased with age in both +/+ and sg/sg. 2. Double label experiments showed that the majority of these receptors were located in the thymus epithelium of the medullary region. 3. In contrast the expression of muscarinic receptors appeared to be developmentally regulated following similar schedules as was described for NAc-neuramimic acid and NAc-neuraminidase (3,4): in +/+ maximum binding activity was expressed in the first week postnatally (P1-4), a crucial time for the selection of thymocyte subclasses. A second peak of propylbenzylcholine binding activity occured in the third week (P19) postnatally, after the T cell repertoire is established. 4. In sg/sg, however, the number of muscarinic binding sites did not exceed background levels in the first week, but were expressed in normal frequences in the third week. Localization studies suggested that muscarinic receptors are expressed on both epithelium and lymphocytes the majority of which were located in the medullary region.

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3. Trenkner, E. (1979), Nature, 277: 566-567.
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210.11 IMMUNOLOGICAL DISORDERS IN THE NEUROLOGICAL MUTANT STAGGERER. E. Trenkner and M.K. Hoffmann*. Dept. of Pharmacology, N.Y.U. Medical Center, New York, NY 10016, Sloan Kettering Institute for Cancer Res., New York, NY 10021.

Inherited diseases are frequently characterized by patterns of multiple disorder. These patterns suggest developmental relationship between affected organs such as time of development sequence of receptor expression (see Rossi, A. and Trenkner, E., these Abstracts) which could lead to simultaneous induction of differentiation. This study describes a model system where two parallely developing systems, cerebellum and thymus, appear to be affected by the single gene mutation staggerer.

The autosomal recessive mutation staggerer located on mouse chromosome 9 is recognized as a neurological mutant because of movement abnormalities and defective cerebellar development. We show here that the staggerer mutation not only affects the development of the cerebellum but also causes developmental and regulatory changes of the immune system: 1) The premature cell surface carbohydrate patterns observed on postnatal staggerer cerebellar cells were observed on staggerer thymocytes and particular spleen cell populations but not on other tissues tested. 2) The peak of particulate neuraminidase activity was delayed in both cerebellum and thymus but not in other tissues. 3) On gross inspection of staggerer we observed a marked delay in the development of the thymus, generally enlarged lymph nodes and undersized spleens. 4) In these mice the frequency of B cell precursors was the same as for normal littermates. 5) They generated antibody forming B cells in vitro and helper T cells in vivo in normal proportions; however, 6) a deficiency in terminating antibody formation in primary responses was noted, suggesting a defect in the regulatory feedback control mechanism.

210.12 NALTREXONE-SENSITIVE SUPPRESSION OF THE IMMUNE SYSTEM'S NATURAL KILLER CELLS BY MORPHINE. Y. Shavit, G.W. Terman, F.C. Martin, R.P. Gale* and J.C. Liebeskind. Depts. of Psychology and Medicine, UCLA, Los Angeles, CA 90024.

Opiates and endogenous opioids have been implicated in modulation of immune function and tumor development. We have previously found that daily exposure to 10 min of an "opioid" form of footshock stress for 4 days rendered rats more susceptible to a mammary ascites tumor challenge. This effect was blocked by naltrexone, suggesting mediation by opioid peptides released by stress. In contrast, daily exposure to a nonopioid form of footshock stress for 4 days had no effect on tumor development. In parallel with these results, we also found that the same paradigm of opioid stress suppressed the cytotoxic activity of natural killer (NK) cells (Science 223, 188, 1984). This effect was blocked by naltrexone, and the nonopioid form of stress did not affect NK activity. NK cells are a subpopulation of lymphocytes that spontaneously recognize and kill certain tumor cells. In the present study, we investigated the effect of morphine on NK cell activity.

Fischer 344 female rats (n=5) were injected with a single dose of morphine (10 or 50 mg/kg, s.c.) or saline. Another group was pretreated with naltrexone (10 mg/kg) 20 min prior to morphine administration. Three hours after morphine, rats were anesthetized and their spleens removed and dissociated into a single cell suspension. Splenocytes were co-cultured with YAC-1 target cells labelled with chromium-51, and NK activity was measured in a 4 hr chromium release assay.

The percent of specific NK cytotoxicity was significantly suppressed by the higher dose of morphine, and this effect was blocked by naltrexone. Thus, morphine at high dose can suppress immune function within 3 hours of its administration, and this effect appears to be mediated by opioid receptors. Several possible mechanisms could explain these results. Morphine might affect NK cells directly, although the occurrence of opioid receptors on NK cells has not yet been determined. Alternatively, morphine might effect NK activity indirectly, for example by modulating activity in the pituitary-adrenal axis or the sympathetic nervous system. (Supported by NIH grant #NS07628 and a gift from the Brotman Foundation).

210.13 THE PRESENCE OF SEROTONIN RECEPTORS ON MURINE LYMPHOCYTES AND MACROPHAGES. T. Roszman*, D.L. Sparks*, J.T. Slevin, W.R. Markesbery*, J.C. Jackson*, and R.J. Cross* (SPONS: L. Middaugh). Sanders Brown Research Center on Aging and the Departments of Neurology and Medical Microbiology and Immunology, University of Kentucky, Lexington, KY 40536.

An interaction between the central nervous system (CNS) and the immune system remains to be proven. In this report we present evidence for the presence of serotonin and spiperone binding to mouse spleen cell membrane preparations devoid of red blood cells.

Saturation isotherms of [^{3}H] serotonin and [^{3}H] spiperone to membrane preparations obtained from spleen cell suspensions containing both lymphocytes and macrophages indicate a single class of saturable binding sites for each ligand. Scatchard analysis of [^{3}H] serotonin binding suggests a ligand affinity (K_D) of 0.85 nM and maximum binding capacity of 0.56 pmoles/10^6 cells. Removal of macrophages from the spleen cell suspension eliminated [^{3}H] spiperone binding to membrane preparations of the remaining lymphocytes. [^{3}H] serotonin bound to these membranes with a K_D of 0.73 uM and a Bmax of 0.53 pmoles/10^6 cells. A membrane suspension of peritoneal macrophages bound [^{3}H] spiperone with a K_D of 15.2 nM and a Bmax of 573 pmoles/10^6 cells.

Evidence suggests that the neurotransmitter serotonin can modulate immune function. Furthermore, [^{3}H] serotonin and [^{3}H] spiperone preferentially bind to specific subpopulations of serotonin receptors (S_1 and S_2 respectively) in neural tissue. It is tempting to speculate that these sites of binding activity represent an interaction between the CNS and immune responsiveness.

This work was supported in part by NIH grants NS17423 (TR), NIA 1 T32 AG00084-01A1 (DLS), NINCDS (TIDA) NS00732 (JTS), and AG02435 (WRM).

211.1 DEPENDENCE OF CORTICOTECTAL CELLS IN AREA 17 OF THE CAT UPON SPECIFIC LAYERS OF THE DORSAL LATERAL GENICULATE NUCLEUS. T.G. Weyand, J.G. Malpeli and C. Lee*. Dept. Psychology, University of Illinois, Champaign, IL 61820.

The dependence of cortical neurons projecting to the superior colliculus (corticotectal, CT cells) upon the integrity of individual laminae of the dorsal lateral geniculate nucleus (LGNd) was examined in the paralyzed, barbiturate anesthetized cat. Specifically, the visual response properties of 32 antidromically identified CT cells in area 17 were examined before, during and after reversible inactivation of retinotopically-aligned regions of layers A or C of the LGNd, or layer 1 of the medial interlaminar nucleus (MIN). Reversible inactivation was achieved by pressure injecting 125 nl of 4 mM cobaltous chloride or 1% lidocaine into one of the geniculate layers. The results indicate that injections into layer C or the MIN were ineffective in blocking visual responses of CT cells. In contrast, inactivating layer A revealed two populations of CT cells: one rendered visually unresponsive and one largely unaffected. Loss of visually driven activity following layer A inactivation does not appear to be simply related to the receptive field properties or antidromic latencies of the individual CT cell. Our sample of CT cells is a heterogeneous group of complex cells possessing wide variability in spontaneous activity, length summation, orientation selectivity and direction selectivity. Nearly all cells tested responded well to a broad range of stimulus velocities.

We conclude the following:

i) The corticotectal pathway from area 17 is composed of a heterogeneous population of neurons. This heterogeneity is reflected both in the receptive field properties of these neurons and in the composition of their geniculate inputs.

ii) Since CT cells do not receive monosynaptic input from the LGNd (Ferster, D. and Lindstrom, S., J. Physiol. 342: 181-215, 1983) and neurons of layers IV and VI are dependent upon layer A (Malpeli, J.G., J. Neurophysiol., 49: 595-610, 1983), we propose that the differences observed in susceptibility of CT cells to blockade of layer A result from differences in intracortical circuitry. Specifically, CT cells whose visual activity is compromised by blocking layer A depend upon layers IV and/or VI while CT cells unaffected by such treatment are supported by other input. The cells of layers II+III must be considered prime candidates for such sustaining input.

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211.2 CORTICAL INPUT TO THE MONKEY LATERAL GENICULATE NUCLEUS IS SPATIALLY STRUCTURED. R.T. Marrocco and J.W. McClurkin*. Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

Recent experiments have presented somewhat conflicting findings regarding the sign of the cortical influence on lateral geniculate nucleus (LGN) cells. Some studies have reported excitatory effects, others showed inhibitory effects, and still others showed both. All of these studies, however, judged the influence "in the negative", that is, by what disappeared following cortical cooling. We have attempted to clarify this issue by combining visual activation of the corticogeniculate pathway (e.g., McClurkin and Marrocco, 1984) with cryogenic blockade.

We recorded from single LGN cells and visually stimulated them with drifting sine-wave gratings restricted in area to 2 deg. The cortical input was assessed by noting any difference in response to gratings before and during cooling. We then rewarmed the cortex and stimulated the region from 2-20 deg around the receptive field with a spinning radial grating, previously shown by us to evoke corticofugal activity. The LGN cell's responses to the drifting sine-wave grating alone, and in combination with the peripheral stimulation were then measured. The cortex was then recooled and the observations repeated in order to determine whether any differences due to the radial grating were cortical in origin. About half of all cells tested showed cortical input.

Our results showed that the cortical input to the central 2 deg was usually of a different polarity than that of the periphery. Excitatory center/inhibitory periphery and vice versa were equally common. In some cells there appeared to be no spatial structure to the cortical influence. These results are similar to those reported by Tsumoto et al (1978) in spite of very different techniques. They suggest that both influences can be found in single LGN cells with the appropriate stimulation. Supported by NSF 82-07531.

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211.3 RECEPTIVE FIELD PROPERTIES OF CORTICOTHALAMIC NEURONS IN VISUAL AREA I OF THE AWAKE RABBIT. Harvey A. Swadlow. Dept. Psychology, U-20, Univ. of Connecticut, Storrs, CT 06268.

The receptive field and axonal properties of 70 neurons which project to the thalamus from layer 6 of visual area I were studied in awake, unparalyzed rabbits. All initial surgery was performed under deep pentobarbital anesthesia. Corticothalamic neurons were identified by antidromic activation following electrical stimulation of the dorsal lateral geniculate nucleus. Stimulating electrodes were also placed in corresponding portions of the superior colliculus in order to differentiate corticothalamic neurons from corticotectal neurons activated in passage via thalamic stimulation. Receptive fields were classified according to the scheme of Murphy and Berman (J. Comp. Neurol., 188: 401, 1979). Eye position was monitored $\pm$ 0.2°.

Antidromic latencies ranged from 1.98 to 34.70 msec (median = 9.0 msec) which corresponded to axonal conduction velocities of 0.49 - 9.55 m/sec (median = 1.93 m/sec). Forty receptive fields were of the simple type (16 simple type I, 24 simple type II). Eighty-five percent of these simple cells were strongly or completely directional selective whereas only 8% were end-stopped. The receptive fields of seven corticothalamic neurons were concentrically organized, and these fields were among the smallest found in the visual cortex (median center diameter = 2.5°). One neuron was directional selective but not orientation selective and six neurons were strongly driven by visual stimuli but had unusual receptive field properties. An additional 16 corticothalamic neurons were vaguely responsive or unresponsive to visual stimulation and most of these cells had very long antidromic latencies (median = 25.9 msec, range = 9.16-34.7 msec). No corticothalamic neurons with complex receptive fields were found.

The above visual and axonal properties of corticothalamic neurons contrast with those of corticotectal neurons in layer 4 and 5 of rabbit visual cortex. (Weyand and Swadlow, Soc. Neurosci. Abstr. 9, 1983). Corticotectal neurons have primarily complex receptive fields and antidromic latencies of less than 2.5 msec.

211.4 RETINOTOPIC ORGANIZATION WITHIN THE LATERAL POSTERIOR COMPLEX OF THE CAT. B. Hutchins and B.V. Updyke, Dept. of Anatomy, LSU Medical Center, New Orleans, LA 70112.

The cat's LP complex contains several zones, each with interconnections to extrastriate areas. Opinion differs concerning the number of zones and their boundaries, however, available data does not wholly resolve these differences. In order to address these issues, we mapped the LP complex electrophysiologically in paralyzed, chloralose-anesthetized cats. The caudal 2/3 of the complex was explored in detail, and limited sampling was done more rostrally. Subdivisions of the LP complex are described using existing terminology (Updyke, J.C.N., 1983; 219: 143-181). Within Pul and LP1-c lines of isoelevation are inclined from anterodorsal to caudoventral with the representation of the upper quadrant located caudally. Representations of the VM are found at the lateral border of Pul and at the LP1-c/LPi border. The Pul/LP1-c border corresponds to a representation of the lateral periphery. Isoelevation lines converge at the Pul/LP1-c border to fan out in either direction toward the VM, indicating expanded representations of central gaze in both zones. Unexpectedly, the representation of the visual field within caudal LPi was found to be folded upon itself. The representation of the VM at the LP1-c/LPi border continues over the dorsomedial surface of LPi and extends for a variable distance between LPi and LPm. As a result, lines of isoazimuth with LPi form tight loops, and the periphery of the visual field is represented in part within the center of LPi. LPi also contains an expanded representation of central gaze. Although LPm has not previously been recognized as visually responsive, we have found a partial representation of the visual field in this zone. At caudal levels the VM is represented at the LPi/LPm border, and peripheral regions of the visual field are represented medially within LPm. Limited mapping at more rostral levels suggests that loci within the lower periphery of the visual field are represented at the LPi/LPm border. Preliminary observations within the rostral third of the LP complex indicate that continuous visual representations extend from caudal to rostral Pul, and from LP1-c to LP1-r, and that elements of the dorsal shell (LPs-d) contain limited representations of the visual field. The present results support subdividing the cat's LP complex into five zones and a heterogeneous shell (Updyke, J.C.N., 1983,) and indicate a more complex role for these subdivisions in integrating visual information and relaying it to diverse cortical areas. Supported by NEI grant nos. F32 EY05703 and R01 EY01925.

211.5 **LAMINAR ORGANIZATION OF CORTICAL CONNECTIONS WITH THE TECTO- AND CORTICO-RECIPIENT ZONES IN THE CAT'S LATERAL POSTERIOR NUCLEUS**

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The cortical connections of the two visual zones within the cat's lateral posterior (LP) nucleus were examined using the anterograde and retrograde transport of WGA-HRP. Microelectrophoretic deposits of this tracer into the main tectorecipient region in the medial aspect of the LP (LPm) revealed reciprocal connections with seven extrastriate cortical areas. These included areas 19 and 21a, the medial and lateral banks of the middle suprasylvian sulcus (CBm and CBl), the superior and inferior banks of the posterior suprasylvian sulcus (DLS and VLS), and an area within the fundus of the posterior suprasylvian sulcus (PS). In each cortical area two distinct populations of cells were labeled: small pyramidal-like cells in layer VI and large pyramidal cells in layer V. In each cortical area the density of back-filled neurons in layer VI was much greater than in layer V. Overlying these filled cells were two bands of anterograde label:a narrow strip in layer I and a significantly wider band centered in layer IV. Deposits of WGA-HRP into the striate-recipient zone in the lateral region of the LP (LPl) resulted in anterograde and retrograde label within the striate cortex, as well as in the following extrastriate cortical areas: areas 19, 20a and b, 21a, CBm, CBl, PS, and an area within the inferior bank and fundus of the splenial sulcus. The laminar organization of extrastriate connections with LPl was very similar to that observed with the LPm. Labeled cells were found in layers V and VI and anterograde label was observed in layers I and IV. These labeling patterns appeared to be qualitatively similar to those obtained following deposits of tracer in the LPm. However, areas 17 and 18 showed a different pattern. Cortical cells projecting to LPl were only found in layer V, whereas the projection from LPl was observed to terminate in layer 1.

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211.6 THE CONNECTIONS BETWEEN AREAS 18 AND 19 AND THE LATERAL POSTERIOR COMPLEX IN THE CAT'S THALAMUS.K.Albus and C.Sanides-Buchholtz*, Dept.Neurobiol.,MPI Biophys.Chem.,Göttingen,FRG.

The lateral division of the lateral posterior complex (LPl) in the cat's thalamus has been claimed to be the only area within LP to receive projections from the visual cortical areas 17,18 and 19 (Updyke,B.V., J.comp.Neur.,173:81, 1977). However, own findings based on the axonal transport of HRP(Albus,K. and Meyer,G.,Soc.Neurosci.Abstr., 7:761,1981) suggested that the connections between LP and visual cortex are more widespread for areas 18 and 19 than for area 17.In order to clarify this matter we have made use of retrograde and anterograde axonal transport techniques. The tracers were Fast Blue (FB,2%), Diamidino Yellow(DY,2%), Nuclear Yellow(NY,2%) and WGA-HRP(5%). Prior to the application of the tracer substances cortical injection points were determined by physiological methods.Survival times were 10-16 days for FB and DY, and 24-36hours for NY and WGA-HRP.Brains were cut at 25µm and in addition to HRP-histochemistry acetylthiocholinesterase histochemistry was routinely carried out.

The results confirm our earlier findings that areas 18 and 19 receive projections from LPl and from a zone medially adjoining LPl, which is tentatively called LPm. As a new finding projections from areas 18 and 19 back to LPm were demonstrated. A precise reciprocity between thalamocortical and corticothalamic projections was seen within LPm, and it could be also shown that the afferent and efferent connections of areas 18 and 19 with LPm are in retinotopic register. By injecting various parts of the visual field representations in areas 18 and 19 the retinotopy in LPm was found to be a mirror image of the retinotopy seen in LPl,with LPm being smaller in an anteroposterior direction than LPl. By comparing the thalamic plots of retro-and anterogradely transported material with the plots of cholinesterase activity a close correspondance was seen between LPm as defined in the present report and LPm as defined on the basis of cholinsterase histochemistry (Graybiel, A.M., and Berson,D.M.,Neurosci., 5: 1175,1980). Since the zone of high cholinesterase activity which is called LPm receives a strong projection from the superior colliculus our results assume that information from the midbrain can be directly relayed via LPm to cortical areas 18 and 19 and /or modulated within LPm by neuronal activity descending from areas 18 and 19.

211.7 CONNECTIONS OF THE VENTRAL ANTERIOR NUCLEUS WITH THE VISUAL CORTEX IN THE CAT R. Rieck Dept. of Anatomy, Tulane University Medical School, New Orleans, LA, 70112.

The ventral anterior nucleus (VA) in the cat has been suggested to be the homologue of the "paralaminar" portion of the ventral anterolateral complex (VAL) in the rodent. Furthermore, the paralaminar VAL has been shown to project extensively to layer I of the visual cortex in the rat. The present study relies upon anterograde and retrograde tracing techniques to determine if a similar projection arises from VA in the cat. Pressure injections of tritiated amino acids (AA) were placed within VA, and the brains were processed according to routine protocols for autoradiography. Similarly, small electrophoretic injections of HRP also were placed within separate regions of the visual cortex, and the brains were processed for HRP visualization.

Following the placement of large AA injections into VA, transported label is located over several neocortical regions. As anticipated by the spread of the tracer into the anterior thalamus, anterograde label is found over the cingulate gyrus. The heaviest label within the neocortex, however, is located over the full extent of the middle suprasylvian gyrus. Rostrally, dense anterograde label is seen over layers I and III-VI in Areas 5 and 7. Similarly, autoradiographic label is seen over layers I and V-VI of Area 21a. Areas 20a and 20b also contain dense label over layer I and layers III-VI. Anterograde label is located primarily over layers V-VI within Areas 17 and 18. Likewise, Area 19 contains moderately dense label over layers V-VI as well as faint label over layer I. Following placement of HRP within Areas 17 and 18, retrograde neuronal labeling within the rostral thalamus is restricted to the central medial and paracentral nuclei. Thus, the anterograde label observed over layers V-VI within Areas 17 and 18 most likely is contributed by intralaminar nuclei rather than by the VA nucleus. In contrast, when retrograde tracers are placed within the caudal part of the middle suprasylvian gyrus, labeled neurons are observed within the dorsomedial segment of the VA nucleus as well as within the intralaminar nuclei. Thus, neurons within the VA nucleus contribute at least part of the laminar terminations seen within Area 21a. In summary, these data suggest that in the cat the projection from VA apparently terminates selectively within the visual cortex rather than projecting to all visual cortical areas as VAL does in the rodent. Supported by NIH grant EY05033.

211.8 RADIOFREQUENCY, BUT NOT KAINIC ACID, LESIONS OF THE MONKEY PULVINAR PRODUCE MASSIVE ANTEROGRADE DEGENERATION IN THE SUPERIOR COLLICULUS. D.B. Bender* and J.S. Baizer (SPON: D.S. Faber). Division of Neurobiology, Department of Physiology, School of Med., University at Buffalo, Buffalo, NY 14226.

Although both physiological and anatomical studies have clearly demonstrated an intimate relation between the primate pulvinar and the visual system, surprisingly little is known of the pulvinar's function. Several studies have reported behavioral deficits following thermocoagulation of the pulvinar. Pulvinar lesions impair color discrimination when the discriminanda are separated from the response sites, they impair discrimination of tachistoscopically presented stimuli, they reduce the rate of gaze shift in visual scanning, and they impair localization of brief peripheral targets. However, these deficits may not have resulted from destruction of the pulvinar itself, but rather from damage to corticotectal fibers passing through the pulvinar.

To evaluate this possibility, and to determine whether kainic acid can be used to destroy pulvinar cells without damaging corticotectal fibers, we compared anterograde degeneration in the superior colliculus following kainic acid and radiofrequency lesions of the pulvinar. Kainic acid injections produced total loss of neuronal perikarya within the inferior and lateral pulvinar. Four to seven days following the kainic acid lesions, terminal and fiber degeneration within the superior colliculus was no greater than that produced by control injections of saline. By contrast, thermocoagulation lesions of the inferior and lateral pulvinar produced dense fiber and terminal degeneration throughout the superficial and intermediate layers of the superior colliculus. Thus, whereas thermocoagulation of the pulvinar severely damages the corticotectal tract, kainic acid lesions spare these fibers of passage.

These results suggest that kainic acid lesions should provide an effective tool for studying the functional significance of the primate pulvinar. Furthermore, the behavioral deficits seen following pulvinar lesions may have told us more about the function of the corticotectal system than they have about the function of the pulvinar.
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211.9 TREE SHREW CLAUSTRUM: LAMINAR ORGANIZATION OF CONNECTIONS WITH EXTRASTRIATE AND TEMPORAL VISUAL CORTICIES. R. G. Carey and T. L. Neal. Div. of Neurobiol., Barrow Neurological Institute, Phoenix, AZ 85013.

The dorsal claustrum has been shown to be connected with vast regions of visual cortex in a variety of mammals. Yet, with the exception of striate cortex, little is known concerning the laminar organization of these connections. In the tree shrew, for example, the claustrum terminates in area 17 in a retinotopic manner in the very cortical layers that receive an ascending thalamic projection (i.e., IV, IIIb, VI and I). It is uncertain how reflective this pattern is for all of visual cortex. In order to examine the laminar organization of claustral connections to other visual areas, small electrophoretic injections of neural tracers (generally WGA/HRP) were made directly into the tree shrew dorsal claustrum.

After claustral injections that resulted in widespead labeled activity in area 17, a number of distinct foci of labeled cells and/or terminals were localized throughout visual cortex. The majority of these foci had a laminar organization similar to that seen in area 17 with the densest terminations occurring in layers IV and VI. The reciprocal projection originated primarily from small- to medium-sized pyramidal cells in layer VI. Further, in a majority of the visual areas, the density of the anterograde labeled terminals and the retrograde labeled cells appeared positively related. However, certain of the visual areas were conspicuous by their lack of symmetry. Area 18, for example, received only a light and scattered (layers IV-I) projection from the claustrum, yet its projection to the claustrum rivaled that of area 17 in magnitude.

A pattern distinctly different from that of the other visual areas was observed in temporal anterior visual cortex. In this area, a number of periodically occurring foci were found in which labeled cells were intermixed with the labeled terminals within layer IV. The majority of these labeled cells were small to medium pyramidals, but a number of medium to large multipolar cells also were labeled.

Thus, our results indicate that while the laminar organization of claustral connections in most of the visual areas is similar to that of area 17, a number of definite differences do exist.

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211.10 THE INFLUENCE OF THE VISUAL CORTEX ON THE RETINAL GANGLION CELLS: QUANTITATIVE DATA. F. Tremblay*, S. Molotchnikoff, and A. Cerat*. Département de Sciences biologiques, Université de Montréal, Montréal, P.Q., Canada H3C 3J7.

The visual cortex (VC) influences light-evoked responses of retinal ganglion cells (RGC) in rats. Previous reports from our laboratory demonstrated that a local (4 mm^2) cryoblockade applied to the VC produces profound modifications of the RGC discharges. The present study is designed to answer two questions: 1) What types of RGC are influenced and; 2) How the response profile is modified, following cortical inactivation. Experiments were conducted on urethane anesthetized hooded rats. Ganglion cell activity was recorded from axons at the optic chiasma level with glass micropipettes. Receptive fields (RF) were studied with stationary images (spots, slits, etc.) generated onto a cathode ray screen positioned 25 cm from the eye. Cells were categorized as concentric, ON, OFF and suppressed-by-light (SBL) according to their response properties when light stimuli were shone within the RFS. The Pearson coefficient (PC) compared control (prior cooling) and test (during cooling) post-stimulus time histograms (PSTH). This comparison indicated that the units most affected by cryoblockade were OFF (41%, N = 29); ON (33%, N = 57) and SBL cells (29%, N = 17). In these RGC the average PC declined to 0.24, 0.36 and 0.27 from 0.66, 0.72 and 0.71 respectively. By contrast only 19% (N = 53) of the concentric cells were influenced by cortical inactivation. The average PC is 0.47 (control = 0.65). As a matter of fact in this class, only two units exhibited strong modifications. Cortico-retinal influences were further studied by using the addition theorem of the chi-squared analysis. The post stimulus time analysis of 500 ms was divided in eight successive equal intervals of 62 ms. This is done to detect during which epoch the test response patterns deviate mostly from the expected (control) profile. The discharges of ON- and SBL cells are modified at the early stages of their response-patterns (increased excitation), while OFF-units exhibited modifications throughout the time of analysis. In conclusion, the visual cortex influences all types of RGC except the concentric class which seems to be spared from cortical blockade. In addition, the VC dampens the inherent oscillatory activity of RGC responses.

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211.11 INTRINSIC AND EXTRINSIC CORTICAL CONNECTIONS OF AREA 17 OF THE PROSIMIAN PRIMATE, GALAGO. J. H. Kaas and C. G. Cusick, Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Surface view patterns of cortical connections of Area 17 of galagos (crassicaudatus) were investigated after single or double injections of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). After fixation, cortex was separated from the brain, artifically flattened, cut parallel to the surface, and processed for HRP. For distances extending 1.5 mm or more, a systematic pattern of patches of labeled cells and terminations in supra- and infragranular layers surrounded each injection site in Area 17. The patches were partially superimposed on foci of high cytochrome oxidase activity. Other connections were with Area 18 (V-II) and with the Middle Temporal Visual Area (MT). A single injection in Area 17 produced several closely spaced foci of labeled cells and terminations in Area 18 and MT. The locations of the clustered foci varied with the injection site in Area 17, so that injections in dorsolateral Area 17 resulted in label in more lateral locations in Area 18 and MT, while dorsomedial injections labeled medial locations in these fields. In both Area 18 and MT, labeled cells were in both supragranular and infragranular layers with a major infragranular component. Additional very sparse label was noted in cortex between Area 18 and MT, providing evidence that Area 17 projects to the dorsolateral visual area or its equivalent in prosimians. The results are consistent with the concept of modular processing of visual information in Areas 17, 18, and MT of galagos, and indicate that Area 18 and MT are the principal cortical receiving zones of information from Area 17.

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211.12 COMMISSURAL PATHWAYS INVOLVED IN THE INTER-HEMISPHERIC CONNECTIONS OF THE ANTERIOR ECTOSYLVIAN VISUAL AREA IN THE CAT. D. Miceli, F. Leporé, R. Ward and M. Ptito*. Groupe de recherche en neuropsychologie, Université du Québec, Trois-Rivières, Québec G9A 5H7

The anterior ectosylvian visual area (AEV) in the cat has been shown to be reciprocally connected with the lateral suprasylvian visual area (LSS) and AEV of the opposite hemisphere (Miceli et al., 1984). Based upon the fact that AEV is topographically isolated from the more classical visual cortical areas which are situated caudally in the hemisphere, the present study was undertaken to determine the commissural system(s) involved in its inter-hemispheric afferent and efferent projections. This was accomplished 1) by examining the orthograde axonal labeling within the forebrain commissures after injection of either of the fluorescent tracers Nuclear Yellow (NY) or Fast Blue (FB) into AEV and LSS, 2) by comparing the retrograde cell labeling in contralateral AEV and LSS following sequential injections of either FB or Evans Blue (EB) and NY into the same regions of AEV made respectively prior to and following a complete transection of the corpus callosum. 1) After LSS and AEV injections, orthograde labeling of glial elements by NY and fibers by FB (Weidner et al. 1983) was observed within the caudal splenium of the corpus callosum, although the AEV-injected tracer was also present rostrally within the commissure. 2) In the corpus callosum transection experiments, compared to the pre-section retrograde cell labeling observed in layers III, V and VI of contralateral LSS and AEV, the post-section tracer injections into AEV failed to label contralateral LSS and layer III of AEV entirely. However, some labeling persisted in layers V and VI of AEV. The results indicate that whereas the AEV-LSS contralateral inter-connections cross over through the corpus callosum, those which are AEV homotopic exhibit both callosal and non-callosal trajectories. The inter-hemispheric transfer of visual information has generally been assumed to involve only the posterior third or splenium of the corpus callosum, however the present results suggest that, with regard to AEV, rostral portions of the callosum and extra-callosal pathways (e.g. anterior commissure) may also be implicated.

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211.13 COMPARISON OF THE STRUCTURAL AND FUNCTIONAL PROPERTIES OF LAMINA V STRIATE CALLOSAL NEURONS WITH THOSE OF CELLS WHICH PROJECT TO THE SUPERIOR COLLICULUS AND LATERAL POSTERIOR NUCLEUS. B.G. Klein, R.D. Mooney, M.F. Jacquin and R.W. Rhoades. Dept. of Anatomy, Univ. of Medicine and Dentistry of New Jersey, School of Osteopathic Medicine and Rutgers Medical School, Piscataway, NJ 08854.

We have used intracellular recording and horseradish peroxidase injection techniques to delineate the structural and functional characteristics of 37 cells in the hamster's visual cortex. Of these, 8 could be antidromically activated from stimulating electrodes in the superior colliculus (SC) or lateral posterior nucleus (LP)[1] and 9 were antidromically driven from the contralateral hemisphere. While the receptive field properties of the two groups of cells overlapped considerably, they were morphologically distinct.

Seven of the 8 cortico-SC/LP neurons had uniform, non-oriented, movement sensitive receptive fields. Two of these were directionally selective. The remaining neuron was a simple cell which exhibited both orientation and direction selectivity. All cortico-SC/LP neurons were large (average somal area 198μm, sd.=87; not corrected for tissue shrinkage) pyramidal neurons with cell bodies located in lower layer V. All had apical dendrites which extended to the pial surface and extensive axonal arborizations in layers V and VI. Axon collaterals which ascended to layer II-III were also visible in 3 of the cells.

Lamina V callosal neurons were more heterogeneous physiologically. Four had uniform circular receptive fields and gave both on and off responses to flashed spots, one was a directionally selective complex cell, one was axially selective, one had a diffuse visual field and two were unresponsive. These neurons had relatively small ($\bar{X}$=133μm^2, sd.=67) somal areas and in only 5 instances did their apical dendrites extend beyond layer II-III. They also had much less extensive axon collateralizations than the cortico-SC/LP neurons.

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[1]These cells will be considered together since most striate cortico-LP cells also project to SC (Rhoades, R.W., Mooney, R.D. and Fish, S.E. Invest Ophthalmol. and Vis. Sci. 22:46, 1982.)

211.14 THE PATTERN OF INTERHEMISPHERIC CONNECTIONS IN THE VISUAL CORTEX OF SQUIRREL MONKEYS. H.J. Gould, III, R.W. Rieck and J.T. Weber. Departments of Anatomy, LSU Medical Center and Tulane University Medical School, New Orleans, LA 70112.

The origins and terminations of interhemispheric pathways were labelled in the visual cortex of adult squirrel monkeys (Saimiri sciureus) by placing multiple injections of horseradish peroxidase (HRP) into the visual cortices of one hemisphere. The hemisphere contralateral to the injections was either cut in the parasagittal plane or removed, flattened between glass plates and cut parallel to the surface. The tissue was processed with the chromagen tetramethylbenzidine according to the protocol of Mesulam ('78).

The flattened material consistently reveals two prominent bands which contain both labelled cells and terminals. The caudal band is located along the 17-18 border. Although this band is oriented primarily in a medial-lateral direction it extends further rostrally on the lateral surface of the hemisphere. The posterior portion of the caudal band is uniformly dense but projections of labelled cells and terminals extend rostrally into Area 18. Examination of the caudal band in the parasagittal plane reveals that the vast majority of labelled cells are located in layer III of Area 18. Clusters of labelled neurons occasionally are found in Area 17 within 150 μm of the 17-18 border. Isolated labelled neurons also are observed in Area 17 up to 1 mm from the 17-18 border. The rostral band forms roughly a mirror-image of the caudal band; it extends caudolaterally from the posterior end of the Sylvian fissure to a point on the lateral surface of the hemisphere where it becomes contiguous with the most rostral extension of the caudal band and then extends rostrolaterally into temporal cortex. Although less distinct than in the caudal band, the rostral band contains a portion that is uniformly labelled and a portion that periodically exhibits extensions. In contrast to the caudal band, the extensions of the rostral band are directed posteriorly. A dorsomedial region and a ventrolateral region between the primary bands, as well as the middle temporal area are relatively free of callosal connections. Although some similarities in callosal patterns exist between the squirrel monkey and other New World primates (Cusick et al., '83) distinct differences exist with respect to Area 17 and the middle temporal area.

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211.15 CORTICAL DYNAMICS OF COLOR AND FORM PERCEPTION. E. Mingolla* and S. Grossberg. Center for Adaptive Systems, Boston University, Boston, MA 02215.

A real-time visual processing theory is used to analyse real and illusory contour formation, interactions between contour and brightness effects, neon color spreading, complementary color induction, and filling-in of discounted illuminants and scotomas. The theory also physically interprets and generalizes Land's retinex theory. These phenomena are suggested to arise from adaptive processes which overcome limitations of visual scanning to synthesize informative visual representations of the external world. Two functionally distinct contour processes interact to generate the theory's brightness, color, and form estimates. A Boundary Contour process is sensitive to the orientation and amount of contrast but not to the direction of contrast in scenic edges. It includes a binocular matching stage that is sensitive to spatial scale, orientation, and binocular disparity, and whose outcome triggers a process of monocular contour completion. These completed contours form the boundaries of monocular perceptual domains. A Feature Contour process is sensitive to both the amount of contrast and to the direction of contrast in scenic edges. It triggers a diffusive filling-in reaction of featural quality within perceptual domains whose boundaries are dynamically defined by the completed boundary contours. The Boundary Contour process is hypothesized to be analogous to interactions initiated by the hypercolumns in area 17 of the visual cortex. The Feature Contour process is hypothesized to be analogous to interactions initiated by the cytochrome oxydase staining blobs in area 17.

References: M.A. Cohen and S. Grossberg, Neural dynamics of brightness perception: Features, boundaries, diffusion, and resonance, 1984; S. Grossberg and E. Mingolla, Neural dynamics of form perception: Contour completion, illusory figures, and neon color spreading, 1984.

211.16 MONOCULAR VERSUS BINOCULAR GANZFELD PRODUCES DIFFERENTIAL UPTAKE OF 2-^{14}C-DEOXYGLUCOSE IN STRIATE AND PRESTRIATE CORTEX OF AWAKE MACAQUE. S.J. Bolanowski, Jr., S. Demeter and R.W. Doty. Ctr. for Brain Res., Univ. Rochester Med. Ctr, Rochester, NY 14642.

Cessation of visual sensation occurs despite continuing stimulation when viewing a stabilized retinal image or Ganzfeld monocularly. It has been commonly assumed, therefore, that: a) such loss of visual experience is retinal in origin, and b) transient inputs generated by saccades are essential for sustained sensation. Such assumptions, however, are negated by the simple fact that vision remains continuous in a binocular Ganzfeld (Bolanowski and Doty, Soc. Neurosci. Abst. 8, 1982). To assay the possible neural substrates differentiating fading from continuing vision in monocular vs. binocular Ganzfelden, we have placed macaques in the same apparatus as used for the psychophysical observations and administered 2-^{14}C-deoxyglucose (2 DG) while they viewed Ganzfeld stimuli for 45 min. Various stimulus configurations were used: monocular vs. binocular; achromatic vs. chromatic; continuous vs. flashed. Depending upon stimulus paradigm, various 2 DG patterns were found in striate and prestriate cortex. For example, a continuous monocular Ganzfeld (green) produced alternating light and dark bands (350-500μm) in layers IV A and IV C of V_1 suggesting ocular dominance demarcations. No patterning was found in layers II and III of V_1 or in prestriate cortex. For the binocular case (e.g., green, 30 sec on-90 sec off), labeling in V_1 was continuous in layer IV and patch-like in layers II and III, the patches being similar in size and distribution to those found on adjacent sections reacted for cytochrome oxidase. Also prominent in this binocular case was substantial uptake of 2 DG in prestriate cortex arranged in strips (400-900μm in width) within layer IV and extending into layers II and III where they were narrower. Since monocular Ganzfelden presented alternately to each eye (e.g., achromatic, 1 cycle/min) do not produce prominent patterns of labeling in prestriate cortex, even though striate cortex possesses patch-like labeling in layers II and III and the expected continuous labeling of layer IV, we suggest that disparate luminances presented to the two eyes can substantially affect information flow between striate and prestriate cortex of the alert macaque. If true, such modulated transmission might underlie the subjective disappearance of a monocularly viewed Ganzfeld.

211.17 SOMATOSENSORY AREA I IS INVOLVED IN VISUAL NEGLECT IN THE CAT. Kenton L. Wertman*, Robert Sinclair*, and Simon Horenstein. Departments of Neurology and Psychology, Saint Louis University, Saint Louis, Missouri 63104.

Ablation of a portion of Somatosensory Area I (SI) in cats produced modification of a visually dependent learned task. As previously reported, ablation of auditory cortex in posterior ectosylvanian gyrus (Ep), or ablation of the medial portion and mesial surface of Area 5, modified preference to Double Simultaneous visual Stimulation (DSS) without affecting lateral preference to unilateral stimulation. Ablation of AI, AII and SII had no significant effect on lateral preference. Clinical cases of sensory neglect are often tri-modal. Therefore, a somatosensory analogue of Ep is likely.

In this study, the functional role of SI was investigated. Six cats were appetitively conditioned in a specially designed "Y" maze (Toga, A.W. et al., Psychological Reports, 40: 1071-4,1977) equipped for visual stimulation. On unilateral stimulation animals were required to move into the sole lighted arm of the maze and on DSS to move into either of two in order to obtain food reward delivered from identical feeding cups at the end of each. Each animal established its own lateralization pattern to DSS. Response to unilateral stimulation was usually appropriate to the side stimulated. After training, sub-pial resection was performed and animals tested from the 1st to the 10th postoperative days. After intracardiac saline-formalin perfusion, brains were removed for anatomic study.

Lesions imposed no apparent postural bias, or preference to unilateral stimulation. In three cats with unilateral SI lesions involving the Coronal and Ansate Sulci, statistically significant differences ($p<.05$) were found between pre and post operative lateral preference in the DSS condition. A substantial portion of SI was destroyed in each case. Some lesions invaded SII. A previous report indicated that lesions restricted solely to SII did not affect lateral preference. In 3 other animals in the present study, lesions restricted to area 5 both lateral and medial to the Lateral Sulcus did not produce significant changes in lateral preference. This latter finding is in agreement with a previous report that lesions excluding the most medial portion or mesial surface of Area 5 did not affect lateral preference, and also provides control for encroachment of the SI lesions into Area 5.

Results suggest that destruction of some portion of SI is sufficient to produce visual neglect.

211.18 AFFERENT CONNECTIONS OF THE CAT'S MEDIAL FRONTAL EYE FIELD. I.M. Jeffers and C.R. Olson*. Department of Psychology, Princeton, NJ 08544

The cat's cerebral hemisphere is thought to contain two frontal eye fields. The more medial of these fields is located on the ventral bank of the cruciate sulcus. The aim of the present study was to identify brain areas projecting to the medial frontal eye field (MFEF), and, further, to establish on connectional grounds the borders and internal divisions of this area. In 8 cats, we placed deposits of distinguishable retrograde tracers (NY and Bb) at separate locations in and around the ventral bank of the cruciate sulcus. Standard charting methods were used.

In its thalamic connections, the MFEF stands out clearly from surrounding prefrontal, limbic and motor areas. Tracers deposited in the MFEF were transported to a medial and ventral division of the ventral anterior nucleus and to a narrow dorsolateral wedge of the mediodorsal nucleus. Tracers deposited closer to the fundus of the cruciate sulcus were transported to thalamic cells having a more ventral and lateral location.

Cortical projections to the MFEF arise from association and high-order sensory areas. These include the posterior cingulate cortex, area 7, the insular cortex, the cortex of the posterior ectosylvian gyrus, and cortex lying within and around the caudal tip of the rhinal sulcus. Occasionally, labeled cells were observed in the lateral bank of the middle suprasylvian sulcus. Tracers deposited closer to the fundus of the cruciate sulcus were transported to more anterior sites within area 7, and topographic patterning was also present in cingulate cortex.

Labeled cells in the claustrum occupied an association zone ventral to the visual and somatomotor divisions.

Our results suggest several general conclusions. (1) The medial frontal eye field is a discrete area occupying much of the ventral bank of the cruciate sulcus and extending onto the adjacent medial face of the frontal pole. (2) Regional specialization must exist within this zone, as indicated by the presence of spatially ordered inputs from several cortical areas and thalamic nuclei. (3) The patterns we have observed are compatible with the view that the cat's medial frontal eye field is homologous to primate area 8. (4) By way of its thalamic afferents, the medial frontal eye field must receive input from the cerebellar dentate nucleus, and may receive pallidal input as well.

211.19 AFFERENT CONNECTIONS OF POSTERIOR CINGULATE CORTEX IN THE CAT. C.R. Olson* and S. Edelstein* (SPON: R. Cholewiak). Department of Psychology, Princeton, N.J. 08544

The "cingular" cortex of the cat, as defined by Rose & Woolsey (1948), occupies the parasplenial gyrus and extends onto the adjacent ventral bank of the splenial sulcus. We have recently become interested in this zone as a result of finding that it projects strongly and topographically both to area 7 (Olson & Lawler, 1982) and to the medial frontal eye field (Jeffers & Olson, this volume). The present study was designed to identify the sources and topography of afferent pathways terminating in this region. Small deposits of distinguishable retrograde tracers (NY and Bb) were placed at separate locations within or near the cingular area of seven cats. The resulting patterns of retrograde labeling, analyzed by use of standard methods, are described below.

Cortex. (1) Area 7 and the medial frontal eye field consistently contained moderate numbers of labeled neurons. More anterior tracer deposits produced more anterior labeling in area 7 and more lateral labeling in the medial frontal eye field. (2) The lateral frontal eye field, insular cortex, and posterior ectosylvian cortex contained moderate numbers of labeled cells, as did a cortical zone apparently including visual area 20 and extending forward into the caudal rhinal sulcus. (3) There was consistent heavy labeling of prefrontal-orbital cortex and light labeling of perirhinal, entorhinal and retrosplenial areas.

Thalamus. Labeled cells occupied a continuous region encompassing the anteroventral, anteromedial and laterodorsal nuclei and the shell zone of the lateroposterior complex (LPs-d and LPs-v). Throughout this region, topography was evident, with more rostral cortical injections labeling more lateral and ventral thalamic cells. It is striking that juxtaposed thalamic nuclei projecting to cingular cortex, area 7 and the frontal eye field are "in register": neurons at a given dorsoventral level within these nuclei project to corresponding, interconnected parts of the three cortical areas.

The results suggest two general conclusions. (1) The cingular area and the anterior thalamic nuclei contain maps of an unknown functional variable which is also represented in area 7, in the medial frontal eye field, and in the principal thalamic nuclei of these areas. (2) The cingular area is suited by its connections to bring sensorimotor processes under the control of prefrontal cortex.

211.20 THE EFFECT OF VESTIBULAR STIMULATION ON THE SPONTANEOUS ACTIVITY OF VISUAL CORTICAL CELLS, J.P. Landolt, S. Reinis D.S. Weiss*. DCIEM, Downsview, Ontario, and Department of Psych., University of Waterloo, Waterloo, Ontario, Canada.

The interaction between the visual and vestibular system is required for an appropriate orientation of the individual in three-dimensional space. At one level, this interaction takes place in the visual cortex where cells can be found which respond to both visual and vestibular stimulation. To study this phenomenon further, the spontaneous unit activity of complex visual cortical cells from Brodmann's area 18 was studied in immobilized, unanaesthetized cats before, during, and after the stimulation of the vestibular system.** The vestibular system was stimulated in several ways: by intravenous injection of deuterium oxide (a noted nystagmogenic agent), by direct galvanic (electrical) stimulation of the labyrinth, by head tilt with subsequent recording in the tilted position, and by sinusoidal movement of the animal in the horizontal plane. In all of these situations, visual cortical cells responded to the vestibular stimulation with an altered rate in spontaneous activity. The direction and shape of the averaged unit response were highly characteristic for each cell, and both increases and decreases in response were observed at the onset, during and following the termination of the labyrinthine stimulation. This indicates that the changes in the spontaneous firing rate are not due to some general arousal effect, but rather, that there is a structured, differentiated response of the visual cells to vestibular stimulation. No such changes were observed during unit recording from non-visual cortical areas. In bilaterally-labyrinthectomized cats, there was no response to deuterium oxide and sinusoidal movement; however,a response was observed to sustained head tilt. This indicates that visual cortical cells are influenced also by a non-labyrinthine sensory input, possibly a proprioceptive one.

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** These experiments conform to the recommendations of the Guide to the Care and Use of Experimental Animals, published by the Canadian Council on Animal Care (CCAC). Furthermore, all of these experiments were approved by the CCAC following their careful analysis and repeated on-site inspections of the anesthetic and immobilization procedures used for animal treatments.

212.1 STEREOSELECTIVE INTERACTION OF AN OPIATE WITH THE NICOTINIC ACETYLCHOLINE RECEPTOR. R. E. Oswald, N. N. Pennow* and J. T. McLaughlin*. Department of Pharmacology, Cornell University, Ithaca, NY, 14853.

A class of opiate drugs, the benzomorphans, bind stereospecifically to the nicotinic acetylcholine receptor from the electroplaque of Torpedo californica. An optically pure, radioactive benzomorphan derivative, (17,18-[^{3}H]-N-allyl-N-normetazocine; (-)[^{3}H]ANMC), was used to characterize this interaction. (-)[^{3}H]ANMC exhibits complex equilibrium binding with at least two independent binding sites having K_D's of 300 nM and 2 μM. The site of higher affinity is decreased allosterically by cholinergic agonists. α-Bungarotoxin has no effect on the binding of (-)[^{3}H]ANMC and does not inhibit the effects of agonists on the binding of (-)[^{3}H]ANMC. This suggests that the allosteric regulation is not mediated by the two well characterized high affinity binding sites for agonists but by another site (or sites) on the receptor molecule. The binding of (-)[^{3}H]ANMC was inhibited by other benzomorphans, with (-) isomers being 4- to 5-fold more potent than (+) isomers. Phencyclidine is capable of inhibiting the binding of (-)[^{3}H]ANMC, and (-)ANMC is capable of inhibiting the binding of [^{3}H]phencyclidine. Affinity labeling with [^{3}H]ANMC was performed using UV irradiation. The α and δ subunits were labeled with the same specificity as the binding of (-)[^{3}H]ANMC to its high affinity site. Trypsin degradation of the membrane-bound receptor indicated that (-)[^{3}H-ANMC labeled a 16 000 dalton C-terminal portion of the δ chain. In contrast, a local anesthetic derivative, 5-azido-[^{3}H]-trimethisoquin, labels an N-terminal 47 000 dalton fragment of the δ chain. These experiments suggest that the binding site for benzomorphans may be a unique site of drug interaction on the acetylcholine receptor. This site is distinct from the acetylcholine binding sites. The binding site of (-)ANMC and that for phencyclidine and 5-azido-trimethisoquin are tightly coupled, but differentially regulated and possibly physically distinct. (Supported by the Muscular Dystrophy Association, NIH grant 1 R23 NS18660-01 AM NEUB, Department of Defense contract DAAK 11-83-C-0049.)

212.2 COATED AND SMOOTH VESICLES PARTICIPATE IN THE INTRACELLULAR TRANSPORT OF ACETYLCHOLINE RECEPTORS. S. Bursztajn, Dept. of Neurology and Cell Biology/Neuroscience Program, Baylor College of Med., Houston, Tx. 77030.

Cultured myotubes contain coated pits and vesicles bearing α-bungarotoxin (α-BTX) binding sites (Bursztajn, S. and G.D. Fischbach, J. Cell Biol. 98:498-506, 1984). We have used α-BTX conjugated to horseradish peroxidase (HRP) and quantitative electron microscopy in order to determine the intracellular pathway of acetylcholine receptors (AChRs) during the internalization process. Cultured rat myotubes were placed at 4°C and incubated with α-BTX-HRP for 2h. Cells were either washed with Hanks basic salt solution (HBSS) and fixed in 2% cacodylate buffered gluteraldehyde, or washed and warmed at 37°C in DMEM for 1 min - 2h and then fixed in 2% gluteraldehyde. Cells were incubated in diaminobenzidine and processed for electron microscopy. Random sections were photographed, and analyzed using a computerized image analysis system. The distribution of coated and smooth vesicles, and T-tubules containing αBTX-HRP reaction product at each time point after warming the cells was determined. The number of coated vesicles/um^2 containing α-BTX-HRP increased two-fold, and the number of smooth vesicles/um^2 containing the reaction product increased three-fold 1h after warming. However, the number of T-tubules/um^2 containing αBTX-HRP did not change throughout the observation period.

A shift in the frequency distribution of smooth and coated vesicles was apparent within 5 min after warming. At this time point, the predominant population of coated vesicles containing αBTX-HRP reaction product was large vesicles 60-120 nm in diameter. At later time points after warming, such a size segregation is no longer observed and both large and small coated vesicles contain αBTX-HRP binding sites. That a shift in vesicle population from coated to smooth occurred is indicated by the observation that within 2 min after warming the predominant population of smooth vesicles containing the reaction product was small vesicles (40-80 nm in diameter). It is only at later time points after warming that the larger smooth vesicles (100-340 nm in diameter) containing αBTX-HRP binding sites become apparent. Coated vesicles were found in continuity with the Golgi cisternae, but these vesicles were not stained. αBTX-HRP reaction product was not observed in the Golgi apparatus, but was present in the lysosomes. Our observations indicate that AChRs are internalized by coated vesicles, which within minutes become smooth vesicles destined for degradation in the lysosome. (Supported by NIH grant NS 17876; the MDA and RCDA to S.B.).

212.3 BROMOACETYLCHOLINE IS AN AFFINITY LIGAND FOR THE ACETYLCHOLINE RECEPTOR OF CHICK CILIARY GANGLION NEURONS. J. Stollberg, D.K. Berg, K. Wan, and J.M. Lindstrom. Dept. of Biology, Univ. of Calif., S.D.; La Jolla, CA. 92093; and The Salk Institute, S.D., CA. 92138.

Chick ciliary ganglion neurons have acetylcholine (ACh) receptors that mediate chemical transmission through the ganglion. In many respects the ganglionic receptors resemble nicotinic receptors in skeletal muscle, but they differ in some ligand binding properties: the ganglionic receptors are not blocked by alpha-bungarotoxin (and may not even bind the toxin) and they appear to have a lower affinity for ACh. Skeletal muscle receptors are known to have a disulfide bond near the active site that can be reduced and covalently labeled with the affinity ligand bromoacetylcholine (BAC). If ganglionic receptors share this property, it may provide a means for labeling the receptor and identifying subunits. We have tested ganglionic receptors for the capacity to be alkylated by BAC at a reduced sulfhydryl moiety.

Ciliary ganglion neurons from 8-day chick embryos were grown for 4-7 days in dissociated cell culture and assayed with intracellular recording for sensitivity to ACh applied to the soma by pressure ejection from a pipet. Membrane conductance changes of about 30 nS were routinely observed in response to ACh at 100 μM. Exposure of the neurons to 1 mM DTT, followed by 10 μM BAC, and then 0.1 mM DTNB and extensive rinsing resulted in a 25-fold reduction in the ACh response. The blockade is specific to the extent that neuronal GABA sensitivity was not depressed by the treatment. Altering the sequence to apply DTT, followed by DTNB, and then BAC and rinsing had no effect on ACh sensitivity, demonstrating that reduction and re-oxidation alone do not account for the blockade, and that exposure to BAC in the absence of reduction does not produce blockade. Moreover, the BAC effect is due at least in part to affinity alkylation since incubation with 1 mM carbamylcholine during the BAC exposure partially protected against the blockade.

These results suggest that the active site topography of the ganglionic receptor is similar to that of the muscle receptor, namely that it has a disulfide bond near the agonist binding site that can be reduced and affinity alkylated by BAC. BAC derivatives may be useful for isolating and characterizing the ganglionic receptor. (Supported by NS 12601, The Muscular Dystrophy Assoc., & The American Heart Assoc.)

212.4 INDUCTION OF EXPERIMENTAL MYASTHENIA GRAVIS IN RABBITS IMMUNIZED WITH AN ACETYLCHOLINE CONJUGATE. M.L.Souan*, M.Geffard*, A.M.Rock* and M.Le Moal(SPON:J.J.Bouyer). Inserm U259, Psychobiologie des Comportements Adaptatifs, rue Camille Saint-Saëns, 33077 Bordeaux, France.

Myasthenia gravis is an auto-immune disease in which a breakdown in tolerance to self-acetylcholine receptor (Ach-R) is reported to result in the production of anti-Ach-R antibodies. The loss in muscular Ach-R activity observed leads to a specific muscular weakness. Our studies show that the induction of experimental auto-immune myasthenia gravis (EAMG) can be obtained after immunization of rabbits with an immunogenic Ach-conjugate. After three booster injections the animals developed a symptomatology that resembled EAMG which became more severe after each successive injection. This immunogenic Ach-conjugate yields:i)anti-Ach antibodies whose specificity was described elsewhere (see poster Geffard et al.); the best affinity is directed against the Ach molecule, but these Ach antibodies can not explain the symptoms of EAMG in rabbits; the immunological mechanism involved requires the development of immunoglobulins specifically directed against Ach-R ii)the existence of the second set of antibodies which can only be understood in considering the anti-idiotypic antibodies could be expected to be complementary to the antigen binding site of the anti-Ach antibodies. The binding of such anti-idiotypic antibodies to the idiotypes has in fact been shown to be inhibited by the antigen Ach-conjugate. Development of the anti-idiotypic antibodies becomes possible only after competition between the auto-anti-idiotypic antibodies and immunogenic Ach-conjugate at the binding site of the idiotypic antibodies. We have demonstrated that the idiotypic antibody site and Ach-R have an equivalent affinity for the Ach-conjugate. This indicates that some antigenic structures of the hypervariable region of idiotypic antibody site must be identical to those of the Ach-receptor site. So, the idiotypic site leads to the development of auto-anti-idiotypic antibodies directed against the Ach-R of rabbit muscle, human muscle and electric organ of Torpedo Marmorata. The specificity of immunorecognition is demonstrated as follows:i) there is no anti-idiotypic antibody affinity for the Ach-esterase active site; ii) a competition exists between either α-bungarotoxin or Ach or Ach-conjugate and auto-anti-idiotypic antibodies on membrane preparations. These results confirm that the nicotinic Ach-R is recognized by a second set of antibodies called auto-antibodies.

212.5 AUTORADIOGRAPHIC COMPARISON OF ^{3}H-NICOTINE, ^{3}H-ACETYLCHOLINE AND ^{125}I-ALPHA-BUNGAROTOXIN BINDING TO RAT BRAIN. P.B.S. Clarke*[1], R.D. Schwartz[2], S.M. Paul*[2], C.B. Pert[2], and A. Pert[1] (SPON: H.C. Holloway). Biol. Psychiatry Branch[1] and Clin. Neuroscience Branch[2], NIMH, Bethesda, MD 20205.

Alpha-bungarotoxin (BTX) has been widely employed to label nicotinic cholinoceptors. However, its status as a CNS probe has been questioned. Recently, high-affinity agonist binding to nicotinic cholinoceptors in rat brain membranes has been demonstrated using either ^{3}H-acetylcholine (ACh) (1) or ^{3}H-nicotine (2). A comparison of dissection studies suggests that the distribution of nicotinic binding sites labeled with ACh differs from that obtained using BTX (1).

Slide-mounted rat brain sections (24 μm thick) were prepared for autoradiography as previously described (3). Consecutive sections, grouped in threes, were allocated for labeling with ^{3}H-D,L-nicotine (3.5 nM), ^{3}H-ACh (10 nM in the presence of 100 μM DFP and 1 μM atropine) or ^{125}I-BTX (1.4 nM). Non-specific binding was assessed in the presence, respectively, of L-nicotine 10 μM, carbachol 100 μM, and L-nicotine 1 mM. Briefly, slides were preincubated, incubated with radioligand, rinsed and air-dried before storage in cassettes against tritium-sensitive film (Ultrofilm). Conditions (ionic composition, temperature and duration of incubation) were optimized for each ligand. Binding to dried tissue sections was quantified by liquid scintillation or gamma counting. Each ligand bound with a single k_D in the low nM range, and was potently displaced by certain nicotinic-cholinergic agents. Racemic ^{3}H-nicotine was displaced in a stereospecific way by cold nicotine (L>D).

The labeling patterns of ACh and nicotine were highly discrete and seemingly identical, with highest densities in interpeduncular n., most thalamic n., superior colliculus, m. habenula, presubiculum, cerebral cortex (I and III/IV), and A9/A10. In striking contrast, BTX binding was dense in both superior and inferior colliculi, cerebral cortex (I and VI), hypothalamus and hippocampus, but was virtually absent in thalamus. These results indicate that ^{3}H-nicotine and ^{3}H-ACh may label the same population of central nicotinic cholinoceptors, which are clearly different from BTX binding sites.

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212.6 ACETYLCHOLINE RECEPTORS CAN ACQUIRE METABOLIC STABILITY AFTER THEY ARE INSERTED IN THE PLASMA MEMBRANE. T.A. Levitt-Gilmour & M.M. Salpeter. Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

Metabolic stability of the acetylcholine receptor (AChR) depends on the presence of the nerve. In the present study we asked whether the nerve can stabilize receptors already inserted in the membrane or whether stabilization requires a modification during some stage of the synthetic process. To answer this question we utilized the facts that 1) receptors inserted in the membrane of denervated neuromuscular junctions have a 1 day half-life just as embryonic receptors do (Nature 291:239-241, 1981), and 2) that if junctions are denervated by crushing rather than cutting the nerve, reinnervation of all fibers occurs synchronously. We therefore labeled the AChRs inserted at denervated junctions and watched their degradation prior to, during, and after reinnervation occurred. We used the sternomastoid muscles of adult mice. Our experimental protocol consisted of: a) saturating the "original" stable junctional receptors (present at the junction prior to denervation) by topical application with nonradioactive α-bungarotoxin (α-BTX) (controls show that >98% of the original AChRs are blocked this way); b) denervating the right muscle by either crushing the nerve, to obtain synchronous reinnervation, or by cutting the nerve and ligating it to prevent reinnervation; and c) labeling the newly inserted receptors by injecting (4.1 μg/100 g body weight) ^{125}I-α-BTX 6 days later. At different times (from 1 to 21 days) thereafter, groups of 4-5 animals were killed by intracardial perfusion and the denervated and innervated sternomastoid muscles removed and cut into 3 pieces, one containing the endplate-band. Endplate-band specific label was determined by subtracting non-endplate-band gamma counts from the endplate-band on a per weight basis.

We found that 1) the receptors from the innervated muscles degraded (as previously shown) with a half-life of 8.5 days; 2) receptors inserted into endplates that were prevented from becoming reinnervated degraded with a half-life of 1 day; and 3) receptors in the endplates denervated by nerve crush first degraded with a half-life of 1 day and then showed a dramatic switch to an 8 day half-life at about 8-9 days after the nerve crush. Electron micrographs of these muscles showed a synchronous appearance of presynaptic nerve terminals during this period. Thus, the nerve can impart metabolic stability to AChRs after they are inserted in the plasma membrane.

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212.7 ACETYLCHOLINE RECEPTOR CLUSTER FORMATION AND EXTRACELLULAR MATRIX PRODUCTION IN CULTURED RAT MYOTUBES IS ENHANCED BY THE IONOPHORE A23187. J. Park,* L.W. Schneider* and S. Bursztajn (Spon: S.A. Berman), Depts. of Neurology and Cell Biology/Neuroscience Program, Baylor College of Medicine, Houston, Texas 77030.

Acetylcholine receptor (AChR) cluster formation in cultured rat myotubes has been shown to be dependent on calcium (Ca^{2+}) concentration of the medium. The size of AChR clusters and their stability is enhanced by high Ca^{2+} concentration. However, the total number of the surface AChRs remains unchanged under these conditions (Bursztajn, et al., J. Cell Biol. 98:507, 1984). To determine whether the ionophore, A23187 can mimic the effect of high Ca^{2+}, we incubated the myotubes in normal Ca^{2+} concentration (1.8 mM) medium and at various concentrations of ionophore (0-200 nM) for 24-72h. The parameters which we have examined are: 1) the number of AChR clusters; 2) total surface AChR number, and 3) acetylcholinesterase (AChE) activity which we have determined by an enzymatic assay and cytochemically on the electron microscopic level. The cultures were labeled with α-BTX-rhodamine, fixed, and observed by fluorescence microscopy. The number of clusters and the number of myotube segments were recorded for each field, and a ratio of the total number of clusters per total number of myotube segments was obtained. The cluster/myotube ratios did not show any significant increase by 24h, at any of the ionophore concentrations tested. However, at 48h a two-fold increase was observed. This increase in cluster/myotube ratio was concentration dependent. The increase was not due to insertion of newly synthesized AChRs since the total number of surface receptors determined by ^{125}I-α-BTX gamma counting did not vary significantly from the control cultures. Electron microscopy of ionophore treated myotubes (200 nM) revealed an amorphous extracellular matrix associated with the plasma membrane. This matrix was frequently seen in the ionophore treated cultures and seldom seen in the controls. Histochemical staining for AChE revealed the reaction product over the amorphous material. However, the total AChE enzymatic activity did not change at any concentration or time of exposure after ionophore treatment. Our results indicate that ionophore A23187 induces AChRs cluster formation without an increase in the total AChR number or AChE activity. *(Supported by NIH grant NS 17876; the MDA and a RCDA to S.B.)*

212.8 THE EFFECT OF PHOSOPHOLIPASE-C ON MAMMALIAN NEUROMYAL JUNCTION. L. M. Konopka* and R. A. Carpenter*, (Spon: A. G. Karczmar). Department of Pharmacology, Loyola University Medical Center, Maywood, IL 60153.

Studies on rat neuro-muscular preparations show that phospholipase-C (PLC) increases the twitch response and Ach depolarization (Watson et al., Pflugers Arch., 363:161, 1976; Harborne et al., ibid., 377:147, 1975). Ohta and Karczmar (Pharmacol. 22:181, 1980), using an amphibian, sciatic nerve/satorius muscle preparation, found a dose dependent increase in EPP amplitudes, mEPP frequency and quantal content. They concluded the PLC exerted both pre and post-synaptic action; the presynaptic effects were Ca++ sensitive.

The present study investigates the effects of PLC upon the mouse neuromyal junction by exposing the phrenic nerve/diaphram preparation to a range of .01 to 1 units of activity/ml (u/ml) of PLC. With a concentration of 0.1 u/ml, the resting membrane potential underwent a biphasic change as depolarization was followed by hyperpolarization. Also, we observed a 25% increase in mEPP amplitude and a 15% increase in EPP amplitude. Finally, there was drastic, presynaptic augmentation (by 50%) of the quantal content (measured by failure method) and of the mEPP frequency (by 60%). These effects were reversed by washout. High concentrations (1u/ml) caused irreversible depolarization of the resting membrane potential and fasciculations; after the washout, we observed a depression of synaptic activity. As these data are consistent with those observed earlier, PLC appears to alter the ionic gating mechanisms through action upon pre and post-synaptic membrane phospholipids.

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212.9 TWO PROTEINS ARE PRESENT IN RAT MUSCLE ENDPLATES AND IN OTHER CELLULAR LOCATIONS. S.C. Froehner. (SPON: M. Marin-Padilla) Department of Biochemistry, Dartmouth Medical School, Hanover, N.H. 03756.

Postsynaptic membrane preparations from the Torpedo electric organ contain a number of proteins that are present in much smaller amounts than the subunits of the acetylcholine receptor or the subsynaptic 43K protein. These proteins may be associated with the postsynaptic membrane in situ or may be contaminants derived from other cellular membranes or from the cytoplasm. Hybridoma technology has been used to identify two proteins that are authentic components of the postsynaptic membrane. Extrinsic proteins were extracted from Torpedo postsynaptic membranes with 10mM lithium diiodosalicylate and were used as immunogen for the production of monoclonal antibodies (mabs). Mabs were screened by an ELISA, immunofluorescence microscopy on rat diaphragm muscle, and immunoblotting techniques. Mab 1351 recognizes an electric organ protein of apparent molecular weight 58,000. When examined by double immunofluorescence microscopy utilizing rhodamine-conjugated αbungarotoxin and fluorescein-labeled antibodies, mab 1351 stains rat muscle endplates very intensely. Extrasynaptic regions of the muscle plasma membrane are also stained but with much less intensity than the endplates. Mab 1403 recognizes a protein of M_r 55,000 with an isoelectric point of approximately 5.2-5.5 and also stains rat muscle endplates although the distribution of the antibody staining is not precisely coincident with the receptor. Intracellular staining of the muscle cells by mab 1403 is prevalent. Since it persists after denervation, synaptic staining with both mab 1351 and mab 1403 is not due to reactivity with nerve terminal components. The results indicate that two minor proteins of isolated Torpedo postsynaptic membranes are highly concentrated at the endplate and are found in other locations in the cell as well.

212.10 A MODEL FOR THE ACETYLCHOLINE BINDING SITE OF THE ACETYLCHOLINE RECEPTOR. W. Luyten, Kennan Kellaris*+ and J. Kyte*+, S. Heinemann and J. Patrick. Molecular Neurobiology Laboratory, The Salk Institute, San Diego, CA 92138. +Department of Chemistry, University of California, San Diego, La Jolla, CA 92093.

The nicotinic acetylcholine receptor (AChR) is the best-studied ligand-gated ion channel. The recent determination of its complete primary structure makes it possible to correlate structure with function. We have built a model for the acetylcholine binding site based upon sequence and biochemical data. The work of Karlin and his collaborators has demonstrated that the quarternary ammonium head of acetylcholine binds to the α-subunit within 1nm of a disulfide bond (1). There is strong evidence that the extracellular domain of the α-subunit consists of residues 1-210 (2). There are four cysteines in the extracellular domain which are conserved between all species which have been examined, i.e., Torpedo, human, calf and mouse. Cys 192 cannot form a disulfide bond with Cys 193. Therefore the disulphide bond close to the binding site must be Cys 128-Cys 142 or one of the disulphide bonds of a double cystine bridge between (Cys 128, Cys 142) and (Cys 192, Cys 193).

CPK models of the regions α125-α145 and α185-α200, as well as the corresponding regions of β-, γ-, and δ-subunits were constructed and folded in antiparallel β-sheet conformation, with β-turns as predicted (3) for both possible cystine arrangements. Despite high local intersubunit homology, only the α-subunit contained a site where acetylcholine fitted well. Various cholinergic agonists, as well as the toxic loops of long and short neurotoxins, could be accomodated by this site. More specifically, Asp 138 and Thr 133 provide the negative charge around the quarternary ammonium group, Ile 131 the hydrophobic interaction, Gln 140 the postulated esterophilic dipole. C10 bis-quarternary compounds can interact with Glu 129, C16 bis-quarternary compounds with Asp 195 and Thr 191. Residues in these positions are conserved across species in the α-subunit but not in the other receptor subunits. In vitro expression and site-directed mutagenesis are being used to test the model.

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212.11 SYNTHETIC NONADECAPEPTIDE OF TORPEDO ACHR GAMMA SUBUNIT RECOGNIZED BY MONOCLONAL ANTIBODIES SPECIFIC FOR CYTOPLASMIC DOMAINS. W.J. LaRochelle* and S.C. Froehner. Department of Biochemistry, Dartmouth Medical School, Hanover, N.H. 03756.

Several models of the transmembrane structure of the Torpedo nicotinic acetylcholine receptor (AChR) have been proposed on the basis of circular dichroism, trypsinization, X-ray diffraction, and hydrophilicity modeling. Monoclonal antibodies (mabs) directed against extracellular and cytoplasmic epitopes of the receptor provide a tool for verification of these models. We have found that two anti-AChR mabs recognize a synthetic nonadecapeptide homologous to a postulated cytoplasmic domain of the gamma subunit. The peptide possesses the sequence Lys-Ala-Glu-Glu-Tyr-Ile-Leu-Lys-Lys-Pro-Arg-Ser-Glu-Leu-Met-Phe-Glu-Glu-Cys (gamma$^{360-377}$) and shares 71% percent homology to a similar region of the delta subunit.

A collection of eleven mabs was assessed for reactivity with the peptide by three techniques. Mabs 264E and 274D previously shown to recognize the gamma and delta subunits bound bovine serum albumin conjugated gamma$^{360-377}$ (BSA-peptide conjugate) and keyhole limpet hemocyanin-conjugated gamma$^{360-377}$ in the ELISA. The binding of both antibodies to native receptor is inhibited by disulfide-linked dimeric peptide more effectively than by monomeric peptide. The dimer inhibited the rate of binding of mab 264E to native receptor half-maximally at 10 uM while the monomer inhibited at 30uM. Similarly, the dimer competed with native receptor at 20nM for 274D while monomer inhibited half-maximally at 68 nM. A control peptide did not interfere with either 264E or 274D binding to receptor. The peptide conjugate inhibited binding to AChR half-maximally at 600nM and 2nM for 264E and 274D, respectively. The binding of neither mab was inhibited by BSA that had been subjected to the conjugation conditions in the absence of peptide. Finally, ^{125}I-BSA-peptide conjugate, and to a lesser extent ^{125}I-peptide, were immunoprecipitated by both mabs. Nine other anti-AChR mabs showed no evidence of reactivity with the peptide or the peptide conjugates by any of these tests.

The recognition of the nonadecapeptide by two mabs whose binding sites have been localized to intracellular domains of the AChR (Froehner etal, J.Biol.Chem. 258: 7112, 1983) suggests that the region gamma$^{360-377}$ is located on the cytoplasmic face of the postsynaptic membrane.

212.12 THE Mr 43,000 POLYPEPTIDE, ν_1, OF AChR-ENRICHED MEMBRANES IS A PROTEIN KINASE. A.S. Gordon, D. Milfay, and I Diamond, Dept. of Neurology, Univ. of Cal. Sch. of Med., San Francisco, CA 94143.

AChR-enriched membranes have been shown to contain 3 alkali-extractable polypeptides of Mr 43,000, ν_1, ν_2, and ν_3 which can be separated by 2-dimensional electrophoresis. ν_1 is a membrane-bound polypeptide having a pI between 7.0 and 7.4 which copurifies with the AChR. Immunochemical studies show that ν_1 is exclusively a post-synaptic membrane protein. We have found that an alkaline-extractable Mr 43,000 polypeptide has an ATP binding site and that the alkaline extract has protein kinase activity. Our results suggest that ν_1 may be a protein kinase. We have used monoclonal antibody (mcAb) to ν_1 to address this question. AChR-enriched membranes were covalently labeled with [α-^{32}P] ATP under conditions where a polypeptide of Mr 43,000 is the only polypeptide labeled. These membranes were solubilized in 0.5% NP-40 and immunoprecipitated with either mcAb or control IgG and Staph. Aureus. We found a covalently labeled polypeptide of Mr 43,000 on autoradiograms of SDS gels of the solubilized immunoprecipitate when mcAb was used. Control mouse IgG showed no immunoprecipitated band. Therefore ν_1 is an ATP binding protein.

In order to demonstrate that the ATP binding protein precipitated by the mcAb is a protein kinase, we show that protein kinase activity is also precipitated from solution by mcAb. pH 11 extract is neutralized and incubated with either mcAb or control mouse IgG and Staph. Aureus is added to precipitate the immune complexes. After centrifugation, the supernatant is assayed for protein kinase activity. We find that the pH 11 extract incubated with anti ν_1 mcAb shows no phosphorylation activity. In contrast, protein kinase activity is observed in the pH 11 extract incubated with control mouse IgG. Therefore, the mcAb must have precipitated the protein kinase and we can conclude that ν_1 is a protein kinase. Since there is only one ATP binding protein present in AChR-enriched membranes, ν_1 is probably the receptor kinase which phosphorylates the AChR.

212.13 METHYLATION OF TORPEDO CALIFORNICA ACETYLCHOLINE RECEPTOR. A.S. Yee* and M.G. McNamee. Dept. of Biochem. & Biophys., Univ. of Calif., Davis, CA 95616.

The acetylcholine receptor (AChR) from Torpedo californica is well characterized both biochemically and functionally. Axelrod and coworkers (Flynn etal.,J. Biol. Chem. 257:9513-9517 (1982)) recently demonstrated that the Torpedo AChR can be carboxylmethylated in vitro by a human erythrocyte methylase and that methylase activity is present in Torpedo electric organ cytosol. We have purified the Torpedo methylase and have studied the effects of methylation on the ligand binding and ion permeability properties of purified AChR in reconstituted membranes.

The use of ammonium sulfate fractionation and gel filtration chromatography gave a 40-fold purification of the Torpedo methylase. As measured with Sephadex G-100, the Torpedo methylase has an approximate molecular weight of 25,000 and can methylate all four subunits with preferential labeling of the α and γ subunits. Methylation of AChR to levels as high as 20 mole% have been obtained using purified methylase.

Since the AChR is the only methylase substrate in the reconstituted membranes, effects of methylation on the function of the AChR can be directly attributed to AChR methylation. There was no detectable effect of methylation on the ligand binding properties of the AChR, as measured by a bungarotoxin competition binding assay. In contrast, methylation to a level of 20 mole% caused a 20% decrease in carbamylcholine-stimulated cation influx, as measured with a manual-mixing ion flux assay using $^{86}Rb^{+}$.

Rapid kinetic studies of the effects of methylation on the initial rates of ion flux are in progress to determine if there exists a one-to-one correspondence between methylation and blockade of ion flux. (Supported by USPHS Grant 13050).

212.14 INHIBITION OF NICOTINIC RECEPTOR MEDIATED ION FLUXES IN RAT SYMPATHETIC GANGLIA BY BGT II-S1 A POTENT PHOSPHOLIPASE. M. Quik. Dept. Pharmacol., McGill Univ., Montreal, Que. H3G 1Y6 Canada.

Previous work had demonstrated that bungarotoxin (BGT) II-S1, a toxin which copurifies with α-BGT, can inhibit nicotinic transmission in rat sympathetic ganglia (Quik & Lamarca, 1982, Brain Res. 238, 385). The mechanism whereby this toxin affects nicotinic function has been further characterized. BGT II-S1 (1 μM) inhibited the carbachol (100 μM) or nicotine (50 μM) stimulated uptake of ^{3}H-agmatine into rat sympathetic ganglia by 73% and 52%, respectively. These responses were also inhibited 90% by d-tubocurarine (100 μM), but unaffected by α-BGT (1 μM) or atropine (10 μM), suggesting that BGT II-S1 affects cholinergic function at a postsynaptic nicotinic site. Binding of physiologically active ^{125}I-BGT II-S1 could be demonstrated to intact sympathetic ganglia; however, the binding could not be displaced by nicotinic agents, suggesting that BGT II-S1 is not interacting at the receptor. Because some neurotoxins produce their effect at the synapse through a phospholytic action, the phospholipase activity of BGT II-S1 was determined. The results demonstrate that BGT II-S1 is a very potent calcium dependent phospholipase. In addition, conditions which abolished the toxin's phospholytic activity prevented its effects on nicotinic transmission and on nicotinic receptor mediated ion fluxes. These include irreversible inhibition of enzymic activity by treatment of BGT II-S1 with p-bromophenacylbromide, as well as reversible inhibition of the phospholipase by substitution of Ba^{++} or Sr^{++} ions for Ca^{++} ions in the physiological medium.

Thus, in rat sympathetic ganglia, BGT II-S1 blocks the nicotinic receptor mediated movement of ions across the membrane. This is probably not due to a direct interaction at the nicotinic acetylcholine recognition site; rather, it may be an ion channel associated effect which is mediated by alterations in the phospholipid environment of the receptor complex or of the membrane. Although BGT II-S1 also has presynaptic actions, in a cultured system of postsynaptic cells, it could prove a useful tool to study the role of phospholipids in neuronal nicotinic receptor regulation.

Supported by the Medical Research Council of Canada.

SENSORIMOTOR INTEGRATION II

213.1 GATING OF LEMNISCAL INPUT AT THALAMIC AND CORTICAL LEVELS DURING CONDITIONED ARM MOVEMENTS IN THE MONKEY. C.E. Chapman, L. Rispal-Padel, M.-T. Parent and Y. Lamarre. Centre de recherche en sciences neurologiques, Département de Physiologie, Université de Montréal, Montréal, Québec, Canada H3C 3T8.

The somatosensory volleys produced by cutaneous stimulation of the forelimb and recorded from the medial lemniscus have previously been reported to be reduced prior to, and during, voluntary movements of that limb. The possibility that afferent input may undergo further gating at more rostral levels of the lemniscal pathway, specifically in the thalamic relay nucleus, ventralis posterolateralis, pars caudalis (VPLc), and in somatosensory cortex (SI), was investigated in the present study.

A macaque monkey was trained to perform rapid flexions of the arm in response to a tone. Low impedance tungsten microelectrodes (20 - 500 kilohms at 1 kHz) were used to make simultaneous recordings from regions of the contralateral VPLc and SI which had homologous cutaneous receptive fields on the arm. Evoked potentials (latency 4.5 - 8.5ms) were recorded in response to electrical stimulation within the receptive field, either through needle electrodes inserted percutaneously beneath the skin or by stainless steel wires held around the superficial radial nerve by a chronically implanted cuff. Control stimuli, given without the movement cue and in the absence of movement, were regularly alternated with the test stimuli which were applied at various delays after the movement cue. The evoked potentials in both VPLc and SI showed parallel changes, decreasing both before and during movement. The evoked potentials which were elicited by stimulation of the forearm began to decrease 50 - 100ms prior to the onset of elbow displacement and showed a maximal reduction to 40 - 50% of the control by the time of the onset of movement. This decrease preceded the earliest electromyographic activity in the operant limb which occured about 40 ms before the onset of movement in this monkey. The delay at which a reduction in the size of the response was first observed and the subsequent maximal reduction covaried, i.e. the earlier before movement onset a reduction occurred, the greater was the observed maximal reduction of that response. Cortical and thalamic regions receiving input from the moving forearm showed the earliest and strongest depression while potentials evoked by stimulation of the dorsum of the hand did not show any modulation with movement. Cortical evoked potentials elicited by stimulation of the thalamus through the recording electrode, under the same experimental conditions, showed no modulation with movement. Similar results were obtained in a second monkey in which VPLc was stimulated with a chronically implanted bipolar electrode.

These results demonstrate that there is no modulation of sensory input from the arm occurring at the level of the somatosensory cortex and further suggest that most of the gating of somatosensory transmission occurs at the level of the first relay, the cuneate nucleus. This latter suggestion is supported by preliminary results obtained from stimulation of the medial lemniscus.

Supported by the Canadian Medical Research Council.

213.2 TOPOGRAPHICAL DISTRIBUTION OF THE CORTICAL AFFERENT CONNECTIONS OF THE ANTERIOR ECTOSYLVIAN SULCUS IN THE CAT J.M. Roda*, C. Cavada* and F. Reinoso-Suárez. Dept. Morfología, Fac. Medicina, Univ. Autónoma, Madrid 34, Spain.

Using electrophysiological techniques, responses to visual, auditory and somatosensory stimuli have been recorded in the cortex of the anterior ectosylvian sulcus (SEsA). In order to elucidate the anatomical basis of the afferent sensory input to the SEsA, we have depicted the location of cortical neurons which project to the cortex of the SEsA using the retrograde axonal transport of horseradish peroxidase (HRP) technique.

Injections of HRP (50% in water) were made in the cortex of the SEsA in eleven adult cats, using a direct visual surgical approach. In one case multiple injections were made involving the whole antero-posterior extent of the sulcus. In the other cases single injections (60 nl) were placed in the rostral, intermediate and caudal portions of the banks and fundus of the sulcus. The sections of the brain were processed using tetramethylbenzidine as chromogen.

The results obtained led us to disthinguish three main sectors in the cortex of the SEsA: 1) The rostral two-thirds of the dorsal bank of the sulcus. This cortical region receives cortical projections from the somatosensory areas SI, SII and SIII, and from the motor cortex. Less abundant connections were found to arise in the anterolateral subdivision of the lateral suprasylvian area (LS) and in area 36. 2) The caudal third of the dorsal bank and the posterodorsal end of the sulcus. This sector receives cortical afferents from the primary, secondary and anterior auditory areas, from SI, SII and SIV, anterolateral subdivision of LS, vestibular cortex and area 36. 3) The lower bank. This sulcal sector receives abundant and bilateral projections from the posterolateral and dorsal subdivisions of LS. The anterolateral and anteromedial subdivisions of LS, areas 20, 21, 19 and posterior suprasylvian, the granular insular area, the cortex of the caudal part of the sylvian sulcus, the secondary auditory area, the caudodorsal part of the suprasylvian fringe and area 36 also project with various intensities to the lower bank of the SEsA.

Our results provide an anatomical substrate for the multiplicity of sensory responses recorded from the SEsA, but suggest that the anatomo-functional organization of this cortical region may be far more complicated than previously reported.

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213.3 MULTIMODAL NEURONS OF MOUSE SUPERIOR COLLICULUS: MAGNITUDE AND SIGN OF INTERACTION DEPEND ON INTERMODALITY DELAY. S.I. Wiener and P.H. Hartline. Eye Research Institute of the Retina Foundation, 20 Staniford St., Boston, MA 02114.

Neurons showing nonlinear multimodal interactions, previously reported in deep layers of both the tectum of rattlesnakes and the superior colliculus of mammals, are probably involved in complex analysis of natural multi-modality objects. We wished to determine how nearly simultaneous the stimuli of two modalities must be to elicit intermodality enhancement or depression. Significant and in some cases maximal nonlinear interaction occurred when one stimulus preceded the other. Unexpectedly, we found neurons which expressed enhancement in one range of interstimulus intervals but depression in another contiguous range.

Enhancement (and depression) is defined here as the percentage by which the response spike count for combined stimulation exceeds (is lower than) that of the single modality response. Modalities included visual, auditory and vibrissal (somatosensory). Extracellular recordings were made from lightly (barbiturate) anesthetized mice. The locations of the multimodal cells were marked by electrolytic lesions and the chemoarchitecture was characterized by cytochrome oxidase and acetylcholinesterase reactions. The recording sites appear laterally in the intermediate white and deep gray layers.

Of the neurons isolated, 10 showed stable response properties long enough to study temporal effects and exhibited enhancement or depression of at least 40%. Among these, the maximal interaction ranged from 80% depression to 240% enhancement. The interstimulus intervals over which enhancement or depression occurred ranged from zero (simultaneous) to 250 msec. Peak interactions occurred mostly at intervals in the range 0-100 msec. Although most of the cells expressed enhancement or depression greater than 20% at zero interval, a few did not; they would have been erroneously classified as unimodal if they had been studied only with unimodal and simultaneously presented bimodal stimuli. Among 4 neurons expressing enhancement and depression in different interval ranges, one will serve as an example: a) a peak depression of 70% occurred at 40 msec (interstimulus interval) b) zero average interaction (and erratic inter-trial variability) occured near 80 msec; c) peak enhancement of 60% occurred at 100-110 msec.

The temporal dependence of multimodal interactions might be expected if a sequence of cues in different sensory modalities has a significant role in collicular-mediated acts.

213.4 UNIT ACTIVITY IN THE PULVINAR NUCLEUS OF A BEHAVING MONKEY SHOWS SENSORY-MOTOR ASSOCIATION. R. Yirmiya* and S. Hocherman* (Spon: Z. Elazar), Dept. of Physiology and Biophysics, Technion Faculty of Medicine, Haifa 31096,Israel

The anatomical connections of the Pulvinar (Pu) with cortical and sub-cortical components of the visual system, and its responsiveness to visual stimuli established its affiliation with vision. However, certain parts of the Pu are known to receive auditory and somatosensory inputs. Recently, some Pu neurons were also shown to fire in relation to intended hand movements.

In view of the functional role of the posterior parietal lobe, with which the Pu connects, and in consideration of the multimodal nature of the Pu itself, we decided to study this structure in a way that would emphasize its non visual properties. The activity of 82 units was recorded from the Pu of a rhesus monkey, performing an auditory discrimination task. In most cases a significant increase in firing rate accompanied the monkey's manual response to the sound. In a number of cases this activity preceded the onset of arm EMG by as much as 100 to 200 msec. Activity associated with hand movement either terminated as the hand reached its target, or declined gradually afterwards. Many units that showed movement related activation also responded to the sound signal. However, this response was relatively small and could be elicited only in a task performance situation.

These findings indicate that the Pu might be involved in processes of sensory-motor association which are not restricted to visual tasks.

213.5 VOCAL FREQUENCY TRACKING OF UNPREDICTABLE FREQUENCY MODULATED TONES: THE TRIGGERING OF BALLISTIC MOVEMENTS. H.B. Nudelman, K.E. Herbrich*, B.D. Hoyt* and D.B. Rosenfield*, Stuttering Center, Dept. Neurology, Baylor Col. Med. Houston Tx 77030.

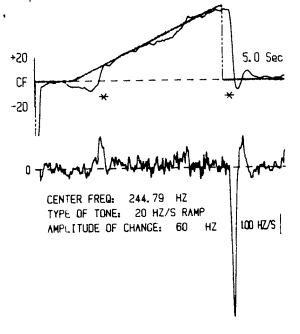
A noninvasive measure of the laryngeal phonatory control system may be obtained by monitoring the fundamental frequency(FF) produced by a person tracking a frequency modulated tone. This FF was calulated, after analog to digital conversion at 10 kHz, by an interactive pattern recognition program using both slope and threshold criterion. The unpredictable inputs were ramps (0.5-100 Hz/sec) and random + and - steps. Below is an example of the data, where stimulus waveform, FF and the rate of change of FF are plotted.

We chose to define separate distinct movements as being bounded by zero crossings of the first derivative of the FF and the points of inflection between these zeros. Movements may then be classified into two categories, ballistic and smooth pursuit, determined by measurements of peak velocity and magnitude. Ballistic movements are (*) on the figure below.

We hypothesize that production of a ballistic movement is triggered by a combination of the magnitude and velocity of the error signal (FF stimulus - FF response). Evidence to support this hypothesis was obtained by measuring the time to the first ballistic movement to ramp inputs. Two results are noted: i. there is a minimum velocity (Hz/sec) required for the production of a ballistic movement, ii. the time to the first ballistic movement produced is inversely related to the input ramp velocity.

This behavior may be modeled with a parallel RC network with the error velocity corresponding to the charging current and the ballistic movement being produced when the circuit is "charged" to a threshold amount of Hz.

Work supported by the Perkins, Bauer and Ariel Medical Foundations.



213.6 THE EFFECTS OF SENSORIMOTOR CORTICAL LESIONS ON SPECIES-TYPICAL BEHAVIOR IN THE MONGOLIAN GERBIL. C. Ellard* and D. Stewart (SPON: C. H. Vanderwolf). Dept. of Psychology, Univ. Western Ontario, London, Ontario, Canada.

The sensorimotor cortex of adult male Mongolian gerbils was removed by aspiration. Following at least two weeks of recovery, the lesioned gerbils and a group of unoperated controls were given a variety of sensorimotor tests and were observed during the performance of a number of species-typical behaviors.

The latency to open and consume sunflower seeds was significantly increased in the lesioned group. Slow motion videoanalysis revealed that this increased latency was due to a deficit in fine digital manipulation.

Lesioned gerbils initiated fewer contacts with female conspecifics and showed deficits in nest-building. Ventral marking, a territorial behavior in which gerbils contact novel objects with abdominal scent glands, was decreased or abolished in the lesioned animals. Forepaw immobility during swimming was abolished.

In contrast, many activities such as walking, running, rearing, grooming and foot-stomping were morphologically normal.

Many of these findings, similar to those reported after equivalent lesions in rats (Vanderwolf et al., 1978), suggest that one role of the neocortex is to inhibit or release specific motor behavior in response to environmental contingencies. The survival of many motor abilities suggests that a great deal of movement programming occurs in subcortical structures.

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213.7 DECREASE IN THE AMPLITUDE OF PHYSIOLOGICAL ACTION TREMOR AND PATHOLOGICAL CLONUS BY TOPICAL ANESTHESIA. R. S. Pozos, W. Mills, and P. Iaizzo, Dept. of Physiology, University of Minnesota, Duluth, School of Medicine, Duluth, MN 55812.

An involuntary high frequency oscillation accompanies slow voluntary movements of the ankle (Pozos, et.al. J. of Appl.Physiol. 52(1):226-230 1982) which has been called physiologic action tremor (PAT). PAT can be enhanced by exercise to produce a physiological clonus which has similar frequency and amplitude characteristics to pathological clonus. Due to these similarities, it was postulated that the control of the damping of PAT might explain both of these overt oscillations of the ankle (Iaizzo and Pozos, J. Appl. Physiol. 53(5)1164-1170, 1982). Recently, it has been reported that topical anesthesia can significantly affect the modulation of the soleus motorneuron pool in humans (Sabbahi, M. et.al. Neurosci. Abst. 298:10 1983). A series of experiments were done to investigate whether the amplitude of PAT or pathological clonus would be decreased by the topical application of an anesthetic. Six normal subjects and two patients who had spinal cord injuries were involved in this study. Surface electromyograms from the leg muscles were recorded simultaneously with motion which was recorded using an accelerometer taped to the dorsum of the patella. These signals were recorded on a FM tape recorder and later analyzed using a MINC-11 computer. The local anesthetic was applied in two different experiments. (1) On the leg that was raised and lowered, and (2) on the contralateral leg. A placebo was applied as in 1 or 2. In the normal subjects, there was a significant decrease ($p<.02$) in the amplitude of PAT in those experiments in which the anesthetic was topically applied to the leg in which PAT was being produced. In the other experimental situations there was no decrease in the amplitude of tremor. Furthermore, the frequency (6-8hz) remained the same. In the case of the spinal cord patients, the topical anesthetic was so effective that clonus could not be induced for approx. 10-15 min. When the clonus was produced, the amplitude was significantly decreased ($p<.02$), but the frequency remained the same. These initial findings indicate that the cutaneous afferents play some role in determining the amplitude of PAT as well as clonus. Since the topical anesthesia had a similar effect on the amplitude of both of the oscillations, these data further support the view that the control of the damping of PAT may explain the increase in the amplitude seen in pathological clonus. Further, the use of topical anesthetics might be of clinical value in decreasing the amplitude of pathological clonus.

CORTEX

214.1 BRAINSTEM PROJECTIONS FROM CORTICAL MOTOR OUTPUT REGIONS (CMORS) TO FACIAL MUSCLES IN THE CAT. R.S. Waters and D.P. Friedman. The Rockefeller University, Laboratory of Neurophysiology, New York, NY 10021 and Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205.

We previously reported the existence of several independent cortical motor output regions (CMORs) in cat cortex where stimulating currents as low as 2-3uA were effective in producing contractions of facial muscles. These regions included motor cortex (area 4), parietal cortex (area 5) and cortex of the anterior ectosylvian sulcus (SIV). While it is well documented that the facial nucleus (FN) provides the common motor output pathway to the facial muscles, little information was available concerning the projection to FN from the CMORs. In the present study we examined the output from each CMOR following injections of tritiated amino acids into a location physiologically defined by its responsiveness to low current microstimulation.

The results demonstrated several parallel pathways to brainstem nuclei, though none of these pathways projected directly to the motor neurons of FN.

Area 4 injections produced most widespread labeling in the brainstem. In the midbrain, terminal fields were found in the magnocellular division of the red nucleus (RNmc), the intermediate and deep layers of the superior colliculus (SC), and the ventral portion of the periaqueductal gray in the region of the interstitial nucleus of Cajal and the nucleus of Darkschewitsch. More caudally, label was found in the tegmental reticular nucleus, central tegmental fields, vestibular nuclei, and a region adjacent to FN.

Area 5 injections produced labeling primarily in RNmc, with an additional smaller projection to the superficial portion of the intermediate layer of SC. By contrast, SIV projections were directed almost exclusively to SC where they terminated in a patch-like pattern in the intermediate and deep layers. With the exception of a projection to the ventral pontine nuclei, no labeling was found caudal to this region.

The pattern of labeling in RN appeared to be similar after area 4 and area 5 injections, while the patterns in SC appeared similar after area 4 and SIV injections. Thus, areas 5 and SIV each appear to share in common one portion of the motor cortical output to the facial musculature and may therefore represent the anatomical substrate that allows for recovery of function following injury to the motor cortex.

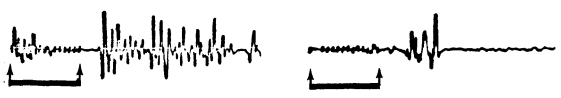
Supported by NIH grant NS-18581

214.2 MICROSTIMULATION MAPPING OF PRECENTRAL CORTEX IN AWAKE BEHAVING MONKEYS. E.M. Schmidt, and J.S. McIntosh. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Monkeys were trained to make alternate wrist flexion and extension movements against different fixed loads or a spring load. Chronic EMG electrodes were implanted in forearm flexor and extensor muscles. The effects of intracortical microstimulation (ICMS) were investigated during the task with trains of 13 to 17 stimuli (0.2 ms biphasic pulses at 300 to 400 Hz) and current levels to 40 ua.

Both excitation and inhibition of forearm flexor and extensor muscles have been observed. When inhibition was produced by ICMS, there was usually rebound excitation following the end of the stimulus train as seen in Fig. 1A. If the monkey was not actively contracting the target muscle during ICMS, inhibition would not be observed but rebound excitation was sometimes present after the stimulus train, as shown in Fig 1B. With only visual observation or muscle palpation, an observer would report excitation of a muscle that was actually inhibited. Thus, EMG recordings while the animal is activating the muscles to be mapped by ICMS is essential for establishing cortical maps showing areas of excitation and inhibition.

Zones of excitation of an individual forearm muscle overlap those of other forearm muscles. The zones are usually quite large, extending up to 6 mm in extent. Inhibitory regions have been found to border excitatory zones. The large representation of an individual muscle in the cortex and the overlap of muscle maps tends to favor the idea that the the cortex is organized in terms of movements where some muscles may be excited while others are inhibited to produce a coordinated movement.



1A 1B

Fig. 1A. Inhibition in palmaris longus at 10 ua. Stimulus artifacts are seen during the period of inhibition. Approximately 14 ms after the end of stimulation there is rebound excitation. Fig. 1B. Rebound excitation in palmaris longus after ICMS during a period when the muscle was silent.

214.3 **CHARACTERISTICS OF CORTICOMOTONEURONAL POSTSPIKE FACILITATION AND RECIPROCAL SUPPRESSION OF EMG ACTIVITY IN THE MONKEY.** R.J. Kasser* and P.D. Cheney. Univ. of Kansas Med. Ctr., Kansas City, KS 66103.

Spike-triggered averaging of rectified EMG activity from multiple forearm flexor and extensor muscles was used to test the output effects of 105 task related motor cortex cells on both agonist and antagonist muscles in two rhesus monkeys trained to perform alternating wrist movements and power-grip. Reciprocal corticomotoneuronal (CM) cells were identified by their postspike facilitation (PSF) of agonist muscle EMG activity and simultaneous postspike suppression (PSS) of antagonist muscle EMG activity in spike-triggered averages. Agonist muscles are those which coactivate with the cortical cell during movement. The purpose of this study was to compare the onset latency, magnitude, and distribution of PSF and reciprocal PSS in forearm flexor and extensor muscles. Of 105 motor cortex cells tested, 53% had no effect on either agonist or antagonist muscles; 33% produced PSF of agonist muscles and 13% produced both PSF of agonist muscles and reciprocal PSS of antagonist muscles. The reproducibility of PSF and PSS was confirmed by computing consecutive STAs and comparing them with randomly-triggered averages of the same EMG activity. The following table summarizes some of the characteristics of PSF and reciprocal PSS.

	Extensors	Flexors	Total
PSFs per reciprocal CM cell†	4.4 (7)	2.7 (3)	3.9 (10)
PSSs per reciprocal CM cell†	1.3 (3)	2.6 (8)	2.3 (11)
PSF onset latency (ms)	6.0	7.9	6.3
PSS onset latency (ms)	14.0	9.3	10.1
PSF peak (% above baseline)	7.3	5.8	7.0
PSS peak (% below baseline)	3.9	4.1	4.1

†Data from cells tested with 5 or 6 agonists and 5 or 6 antagonists. Parentheses indicate number of CM cells.

Onset latencies were measured at the point where the postspike effect exceeded the baseline by two standard deviations. Reciprocal PSS is longer in latency, weaker in magnitude and more narrowly distributed across forearm muscles than PSF from the same CM cells. These results are consistent with a less direct synaptic coupling for PSS than PSF, probably involving a spinal inhibitory interneuron.This work was supported by NIH grant #NS-16262 and NSF grant #BNS 82-16608

214.4 MOVEMENT VS LOAD DIRECTION INTERACTIONS IN AREA 4 ACTIVITY. M. L. Hyde* and J. F. Kalaska. (SPON: H. Jasper). Centre de recherche en sciences neurologiques, Faculté de médecine, U. de Montréal, Qué. H3C 3J7.

The influence of the direction of movement and of the direction of applied loads on area 4 cell activity is being investigated. Monkeys make movements in eight different directions from an actively-maintained central starting position (Georgopoulos et al., 1982). The task is performed with no load, or while compensating for a load applied in one of eight directions, each paralleling one of the movement directions (i.e. 9 x 9 treatment table). For detailed analysis we selected 67 cells which were related to movements of the shoulder joint or girdle and which showed a significant variation in discharge with movement direction (F-test; $p<0.05$). Variation in cell activity with movement direction tended to describe a bell-shaped curve, in which discharge was maximal during movements in a preferred direction (PD) and gradually less during movements increasingly different from the PD.

When the monkey held its arm at the start position, loads applied in different directions significantly affected the discharge of 62/67 cells (F-test; $p<0.05$). This modulation also was continuously graded, usually describing a bell-shaped curve. The ratio of discharge rates of the peak to the trough of this curve is a measure of the influence of different load directions on the cell activity. The median ratio observed was 3.4:1 (range 1.3:1-442:1). The discharge in relation to movement for 55/67 cells was modulated significantly by loads (F-test; $p<0.05$). For each movement direction, the variation in cell activity with different load directions was also continuous and bell-shaped. The median ratio of modulation of activity during movement, caused by loads in different directions was 3.04:1 (range 1.1:1-61.5:1). The load direction which produces the maximum enhancement of cell activity compared to the no-load condition will be called the maximum load axis. The maximum load axis tended to be opposite to the preferred direction in 54 cells, which is the expected relationship.For 8 cells, however, the maximum load axis was approximately perpendicular to or in the same direction as the PD.

The effect of direction of movement and of load on cell activity was not independent. Interaction between these two parameters often was evident as a change in the relation of cell activity to movement direction under different load directions, which produced small but significant shifts in the cell's preferred direction compared to the no-load condition. All of these properties of cell behavior (including continuous variation with direction of movement and load, and movement/load direction interactions) were also seen in EMG recordings of the activity of shoulder muscles in the task. The movement/load interactions therefore might result from shoulder biomechanics. Moreover, the observation that continuous variations in cell activity occur with both changes in movement direction and in direction of applied load supports the argument that they are alternate expressions of the same process, the control of the patterns of muscle activity by the motor cortex. (Supported by an MRC Scholarship and MRC grant MA-7693).

214.5 AREA 5: RELATIVE EFFECT OF MOVEMENT DIRECTION VS DIRECTION OF APPLIED LOAD. J. F. Kalaska, M. L. Hyde*, and R. Wechsler*. Centre de recherche en sciences neurologiques, Faculté de médecine, U. de Montréal, Qué. H3C 3J7.

Previous experiments suggested that one function of area 5 may be to monitor current limb posture and movement, as part of a distributed system controlling visually-guided movements. When movements of constant trajectory are made using different patterns of muscle activity, would area 5 cell discharge remain relatively unaffected, and thus reflect the constant movement trajectory, or would it vary due to muscle activity-dependent changes in the feedback from the limbs? A preliminary investigation of this question has been made in area 5 of the monkey. The task involves movements in 8 different directions from an actively-maintained central starting position, while compensating for loads applied in a range of different directions (Hyde, M.L. et al., Neurosci. Abstr., 1984). Thirty-one cells which were related to movements of the shoulder and which showed a significant variation in discharge with movement direction (F-test; $p<0.05$) were selected for detailed analysis. As was observed in the motor cortex (ibid.), the variation in cell activity with different movement directions tended to describe a bell-shaped curve, with maximal discharge occurring during movements in one preferred direction (PD).

When the monkey held its arm at the start position, the presence of loads applied in different directions had a significant effect on the discharge of 26/31 cells (F-test; $p<0.05$). This modulation was continuously graded with load direction and described a bell-shaped curve. The median modulation ratio observed (discharge rate of the peak of the curve:discharge at the trough) was 1.6:1. Thus the presence of loads in different directions during the hold period had a significantly weaker effect on area 5 activity than was observed in area 4 (K-S test; $p<0.001$). The discharge in relation to movement of only 12/31 cells was significantly modulated by loads in different directions, which was a smaller proportion than in area 4 (χ^2 test, $p<0.001$). The median ratio of modulation of activity during movement was 1.25:1, which is also a significantly weaker influence than observed in area 4. The maximum load axis (ibid) tended to be opposite to the PD in only 11/26 cells; the maximum load axis was approximately perpendicular to the PD for 8 cells and was about the same as the PD for 7 cells. These "unexpected" relations between the maximum load axis and PD were significantly more common in area 5 than in area 4 (χ^2 test; $p<0.001$). These observations are in partial agreement with a previous study of this question (Jennings, V.A. et al., J. Neurophysiol. 49: 1216, 1983). Differences may be due to differences in the properties of cells related to the shoulder as compared to wrist.

In summary, these preliminary data suggest that the variation of cell discharge in area 5 during a movement is primarily related to the trajectory of the movement, and is only partly a reflection of the muscle activity producing the movements. (Supported by an MRC Scholarship and MRC grant MA-7693).

214.6 FRONTAL LOBE INPUTS TO THE CAUDAL HAND REPRESENTATION OF THE PRIMATE MOTOR CORTEX. P.L. Strick and J.B. Preston. Depts. of Physiology and Neurosurgery, SUNY-Upstate Medical Center and VA Medical Center, Syracuse, NY 13210.

In previous studies we demonstrated that the primary motor cortex of the squirrel monkey has two spatially separate representations of the hand (J. Neurophysiol., '82a,b). In the present study we have analyzed the input from premotor areas in the frontal lobe to the caudal hand representation. We have focused on the caudal representation because the region comparable to our caudal zone is buried in the central sulcus of other primates and has not been studied extensively. Also, the connections of the caudal hand representation seemed particularly interesting because neurons in this zone receive input almost exclusively from cutaneous receptive fields located on the volar surface of the hand.

Area 4 of squirrel monkeys (Saimiri sciureus) was mapped using single unit responses to somatosensory input and intracortical stimulation to define the boundaries of the rostral and caudal hand representations, as well as the boundary between areas 3a and 4. Once identified, the caudal zone was injected with 0.01ul of 2% WGA-HRP. After a 4 day survival period, animals were anesthetized and perfused with phosphate buffered aldehydes. Tissue sections were processed using the TMB technique (Mesulam, '78).

In 2 animals, WGA-HRP was confined to the caudal hand representation. In these animals, 2 spatially separate regions in the frontal lobe rostral to the motor cortex contained substantial numbers of labeled neurons. The first region was located in a part of area 6 rostral and medial to the injection site. The second region was located on the mesial wall of the hemisphere ventral to the cingulate sulcus at approximately the same anterior-posterior level as the first region. In those animals where WGA-HRP spread to include the rostral representation labeled neurons were found at additional sites in the frontal lobe.

These results emphasize that premotor areas in the primate frontal lobe have substantial projections to at least the caudal hand representation of the primary motor cortex. Our observations suggest that the premotor areas have a significant involvement in the control of distal limb movements, instead of, or in addition to, their proposed involvement in the control of postural adjustments. Supported by USPHS NS 02957 and VA Medical Research Service.

214.7 DIFFERENCE OF TERMINAL SITES BETWEEN THALAMO-CORTICAL AND CORTICO-CORTICAL FIBERS ON GOLGI-IDENTIFIED MOTOR CORTICAL NEURONS IN THE CAT. M. Ichikawa*, K. Arissian*, and H. Asanuma. The Rockefeller Univ. New York, N.Y. 10021

We have reported that microstimulation within the sensory cortex elicited EPSPs primarily in stellate type neurons whereas VL stimulation elicited EPSPs both in stellate and pyramidal neurons in the cat motor cortex (Asanuma et al. Neurosci. Abst. 1983). To examine the structural basis of these physiological observations, details of the terminal connections of cortico-cortical and thalamo-cortical fibers on pyramidal and stellate type neurons in the cat motor cortex were studied using rapid Golgi, electron microscopic and degeneration techniques.

Cortico-cortical connections were examined using 11 pyramidal and 12 stellate type cells which were identified by rapid Golgi technique. The stellate type neurons located in layer III received many degenerating terminals (average 6.3) and the majority of these (95%) were found on the proximal dendrite or the cell body whereas the pyramidal neurons received fewer terminals (average 2.3) and these were located on more distal dendrites or on dendritic spines. The majority of these synapses were of the asymmetric type.

Thalamo-cortical connections were examined using 9 pyramidal cells and 9 stellate type cells. The pyramidal cells received many terminals (average 7) and these were found on the basal as well as apical dendrites and on dendritic spines.The stellate cells received fewer terminals (average 3) and these were located primarily on dendritic shafts near the cell body. The majority of these synapses were of the asymmetric type.

The results are in agreement with the physiological observations that cortico-cortical fibers make more powerful synapses on stellate type neurons whereas thalamo-cortical fibers make effective synapses on both stellate and pyramidal neurons.

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214.8 CALLOSAL SYNAPSES WITH CALLOSAL PROJECTION NEURONS IN THE MOUSE PRIMARY MOTOR CORTEX. L.L.Porter*and E.L.White.(SPON: R.L.ST.Marie).Boston Univ. Sch. Med.,Boston, Ma. 02118.

Callosal projection neurons in the mouse primary motor cortex (MsI) were identified as pyramidal neurons in layers II-III and V. Previously, we have shown that cells located in both of these cortical tiers receive input from the homotopic area of the contralateral cortex to which they project (L.L.Porter and E.L.White, Neur.Lett., in press). In this study we examined the apical dendrites of callosal projection cells in both cortical layers to determine the distribution and amount of callosal input received by the two groups.

Retrograde labeling with horseradish peroxidase (HRP) was coupled with lesion induced degeneration to identify neurons which project to the homotopic area of contralateral MsI and the axonal projection originating in this location. Eight mice received HRP injections into the vibrissal region of MsI, followed, the next day, by aspiration of the same area of cortex. A post-lesion survival time of four days was allowed as at this time most callosal axon terminals are in a similar stage of degeneration and are easily identified. Thus, four days after lesioning, the mice were perfused, their brains removed and the unoperated hemisphere was cut in the coronal plane and reacted for HRP. Seven labeled cells from each tier were prepared for electron microscopy. The portion of each neuron's apical dendrite which passed through the superficial cortical layers, where most callosal afferents terminated, was cut in an unbroken series of thin sections. Electron micrographs were taken of each labeled profile in each section. The apical dendrite was graphically reconstructed by fitting tracings of the micrographs onto one final montage which included all spines and synapses. From the reconstructions we determined; 1)the number of synapses formed by each cell with callosal axon terminals 2)the distribution of callosal input on the dendrites and 3)the distribution of normal synapses. By sampling small volumes of neuropil, we also compared the amount and laminar distribution of degenerating terminals in the neuropil surrounding the labeled dendrites with that of the distribution and amount of callosal input onto both groups of cells. The amount of callosal input received by the cells in layers II-III and the distribution pattern of callosal input on their dendrites suggested that afferents from contralateral MsI may play a greater role in modulating the activity of the more superficial callosal projection neurons than that of cells in layer V.

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214.9 CELLS IN INTERNAL GRANULAR LAYER OF MACAQUE PREFRONTAL CORTEX PROJECT TO THE CAUDATE NUCLEUS. T. ARIKUNI* and K. KUBOTA (SPON : M. SAKAI). Dept. of Anat. Osaka Univ. Med. Sch., Osaka 530, Japan ; and Dept. of Neurophysiol., Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

Although cells in the internal granular layer of the cerebral cortex give rise to intracortical or callosal connections in the monkey, it is unknown whether they project to subcortical structures. To examine such a possibility, horseradish peroxidase (HRP) was injected into the part of the caudate nucleus which receives afferents from the prefrontal cortex. After callosotomy, HRP polyacrylamid gel (0.1 µl, 10%, Sigma VI) was injected, under direct vision, unilaterally into the caudate medial head in five Macaca fuscata, four Macaca mulatta, and one Macaca irus monkeys. After survival time of 3 or 4 days, the brains were removed and cut transversely at 50 µm thickness with a freezing microtone. Sections were treated with TMB and lightly counterstained by 1% neutral red solution. Injection sites were stained by DAB method.

In the lateral, medial, or orbital surfaces of the ipsilateral prefrontal cortex of all examined monkeys, HRP labeled cells appeared in the cortical layer IV, or the internal granular layer. They were of pyramidal, stellate, or fusiform shape and their size was small (5 to 10 µm). The most commonly labeled were pyramidal. In the most densely labeled case, labeled cells constituted about 20% of the total population of cells in the layer IV. Smaller round-shape cells of small size (less than 5 µm) were never labeled. A small number of labeled cells was also observed in the same layer of restricted regions of the contralateral prefrontal cortex.

Prefrontal projection to the caudate nucleus was also observed in cells of other layers; small pyramidal cells of layer II (5-7 µm, sporadic), medium-sized pyramidal cells of layer III (10-15 µm, considerable), medium-and large sized pyramidal cells of layer V (15-25 µm, dense), and small pyramidal cells (10 µm), and fusiform cells (6 µm x 15 µm) of layer IV (moderate).

It is concluded that in the monkey the cells in the internal granular layer of the prefrontal cortex send their axons bilaterally to the medial head of the caudate nucleus.

214.10 ELECTROPHYSIOLOGY AND MORPHOLOGY OF FOUR CLASSES OF PREFRONTAL NEOCORTICAL NEURONS STUDIED IN VITRO. A.A. Grace and R. Llinàs. Dept. Physiology & Biophysics, NYU Med. Ctr., New York, NY 10016.

Using in vitro brain slice techniques (Llinàs & Sugimori, J. Physiol. 305: 171,1980), we have studied the anatomy, firing pattern and ionic mechanisms underlying action potential generation in four classes of neurons in the medial prefrontal cortex of the guinea pig. The principal cell type is the large (25-40 um) pyramidal neuron, located in layers III-V and characterized by a divergent basal dendritic field, with an apical dendrite extending to layers I-II before bifurcating. Both the apical and basal dendrites contain dense spinous processes. The axon branches locally with occasional recurrent collaterals, as well as projecting toward the white matter. This cell type is capable of firing in two patterns: 1) burst firing at hyperpolarized membrane potentials, and 2) repetitive firing at resting or depolarized membrane potentials. TTX and TEA treatment demonstrated that bursting is driven by a low threshold Ca^{++} spike (LTS) which is inactivated at resting levels (as in the inferior olive, Llinàs & Yarom, J. Physiol. 315: 549,1981), whereas a series of large amplitude (>30 mV) high-threshold Ca^{++} spikes (HTS) trigger repetitive spiking. These cells are unresponsive to dopamine but respond to norepinephrine and db-cAMP with hyperpolarization and an attenuation of firing accommodation in response to a depolarizing pulse (as in hippocampus, Madison & Nicoll, Nature 299: 636,1982). A second class of smaller (<15 um) pyramidal-like cells was penetrated in layers V-VI. The basal dendrites were sparsely spinous, and the apical dendrite ramified in layer IV. These cells fired repetitively and exhibited HTS.

The third class of cells was the stellate cells of layers II-III. These cells have spine-covered dendrites which arise from the soma in 3-4 fascicles and branch profusely within these layers. The axon collateralizes locally and often forms dense plexuses of boutons presumably around neighboring cells. These cells also have LTS and HTS, although they generally fire only a single HTS or widely spaced HTSs, unlike the repetitive HTS generation of the large pyramidal cells. This difference is reflected in their rapid accommodation to a depolarizing pulse. The stellate cells respond to dopamine with a hyperpolarization. The fourth cell type is a small spidery cell in layers I-II. This cell has many thick dendrites emerging from the soma and branching locally, with less dense spinous processes. The ventrally directed axon emits a sparse number of recurrent collaterals. This cell has a fast (<1 msec) spike and accommodates rapidly to depolarization. A rapidly inactivating LTS is present and the HTS is of a shorter duration than was observed for the other cell types studied. (Supported by NIH grants NS13742 & NS07124)

214.11 SELECTIVE ACTIVATION OF PERIPHERAL NERVES VIA TRANSCRANIAL STIMULATION OF THE MOTOR CORTEX IN THE CAT. R. Rumpf, W. J. Levy, M.D., M. McCaffrey,* C. Kline*, and D. H. York. Division of Neurosurgery, University of Missouri School of Medicine, Columbia, Missouri 65212

The motor cortex of cats can be activated by not only direct stimulation but transcranial stimulation between an electrode on the overlying scalp and one in the hard palate, directing current down through the motor cortex. This technique activates the corticospinal tracts and can result in contralateral limb movement (Levy, McCaffrey, York and Tanzer, NEUROSURGERY, August, 1984). This signal travels primarily in the lateral corticospinal tract with some components in the ventral cord, probably the anterior cortical spinal tract and local circuit activity in the ventral gray. It is a potentially useful experimental tool as well as a clinical diagnostic technique (Levy, York, McCaffrey and Tanzer, NEUROSURGERY, August 1984).

We have studied the question of whether selective activation of a portion of the motor cortex, resulting in selective stimulation of the peripheral nerves in only a single limb, can be accomplished by transcranial stimulation. This mapping of the motor cortex is well established as a technique with direct stimulation. We used adult cats anesthetized with Ketamine and Rompun, paralyzed with Pavulon, 0.1 mg per kg. Animals were maintained on a respirator with blood gases determined when necessary. Evoked potentials were recorded from the spinal cord epidural space at two sites, from the sciatic nerve in the hind limbs and in the radial nerve in the forelimbs at one or two sites each on a Cadwell 7400 evoked potential signal averager. The stimuli were delivered by either a Grass S88 through a SIU7 or the Cadwell 7400 stimulator by an electrode, 2 mm in diameter, moved over the scalp in line with the motor cortex by a micromanipulator. The other electrode was a 2 mm diameter electrode placed up against the hard palate in the mouth. Stimulation between these two electrodes with the scalp electrode anodal (using 10 to 20 milliamperes for 500 microseconds) resulted in a traveling wave in cord and peripheral nerves with a velocity of approximately 60 meters per second. Moving the transcranial electrode across the scalp it was observed that lateral stimulation activated contralateral forelimbs and medial stimulation activated contralateral hind limbs. Some adjustments in the palate electrode were also helpful. This selective activation indicates that the current used in the transcranial stimulation is relatively well localized on the surface of the brain and that activation of only a portion of the descending pyramidal tract in the spinal cord is possible in such a stimulation modality. This should offer experimental and clinical methodologies for investigating the central and peripheral nervous system.

SPINAL CORD AND BRAINSTEM II

215.1 CHARACTERISTICS OF VENTRAL ROOT EVOKED POTENTIALS IN SACRO-COCCYGEAL SEGMENTS OF THE SPINAL CORD ELICITED BY STIMULATION OF INTACT AND SECTIONED DORSAL ROOTS. P. Pacheco*, M. Martinez* and B. Dubrovsky. Inst. Inv. Biomed., UNAM Mexico, Allan Memorial Inst., McGill University, Montreal, Quebec, Canada H3A 1A1.

Segmental sensory motor organization of pelvic floor and tail musculature is mediated through the sacrococcygeal regions of the spinal cord. We investigated the morphological characteristics: latency, amplitude and components of evoked potentials, in sacrococcygeal ventral roots in response to (1) electrical stimulation of their intact dorsal roots and (2) by stimulation of the proximal end of the sectioned dorsal roots. Experiments were performed in cats. Under ether anaesthesia, a trachea cannula was positioned and the spinal cord was sectioned between the C_1 and C_2 levels. Animals were put under artificial ventilation and ether was discontinued. Stimulation of dorsal roots from S_1 to C_{x5} all elicited monosynaptic ventral root responses. Criteria for monosynaptic responses were latencies shorter than 1.2 msec., measured from the time of arrival of the afferent stimulating volley recorded monofocally via a silver ball electrode at the site of spinal entry of the dorsal roots; high frequency following, and constant latency. No monosynaptic potential could be evoked in C_{x6} ventral root. Intensities for dorsal roots stimulation, expressed in terms of multiples for the threshhold, T, (minimal current required to elicit a visible afferent volley), were set at 4T in all cases. Polysynaptic evoked potentials could be recorded from all ventral roots S_1 to C_{x6}. The mono and polysynaptic potentials from all recorded ventral roots had individual characteristics which were systematically observed in all the twelve animals studied. Thus, e.g. both S_2 and C_{x2} monosynaptic reflex components were of low amplitude (60 μV or less); but while S_2 had three clearly defined polysynaptic components at 3.5, 4.5 and 6 msec., the Cx2 polysynaptic potential was present as one homogenous component at 3 msec. After section of dorsal roots the proximal end of the roots was again stimulated. In all cases, from S_1 to C_{x5}, stimulation of the severed dorsal root elicited a monosynaptic response whose amplitude was 2- to 4-fold larger than before section. The increase of the monosynaptic reflex response was observed immediately after section of the roots and remained stable up to the end of the experiment, 3-4 h later. Similar amplitude changes were observed with tetanic priming stimulation of the roots.

215.2 COMPARATIVE STUDY OF RENSHAW CELL UNIT AND FIELD POTENTIALS. W.G. VanMeter and E.J. Willetts*. USAMRICD, Aberdeen Proving Ground, MD 21010, Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Iowa State University, Ames, Iowa 50011.

Responses of Renshaw cell field potentials (RFP) and Renshaw cell unit potentials (RUP) to antidromic stimulation of the ventral roots of lumbar spinal segment 7 (L-7) were compared in adult male and spayed female DIAL anesthetized cats of mixed breed.

Extracellular recordings by conventional methods show: (i) a delineated threshold for both RUP and RFP, (ii) that with stimulus intensities greater than threshold, the RUP responds with an increased duration in spike discharge (to a maximum), (iii) that with stimulus intensities greater than threshold, the RFP responds with an increased amplitude (to a maximum),(iv)both RFP and RUP are depressed by increased stimulus frequencies, (v) both RFP and RUP show post-tetanic depression and post-tetanic potentiation.

Unlike the RUP, the RFP can be recorded for several hours which renders it useful to study onset and recovery from systemic drug application. A pharmacological analysis of these potentials reveals that (i) the centrally acting anticholinesterase diisopropylfluorophosphate (DFP) (3.0 mg/kg i.v.), (ii) atropine sulfate (2.0 mg/kg i.v.) and (iii) mecamylamine (2.0 mg/kg i.v.) alter the RFP and RUP in a similar manner consistent with a central nicotinic cholinergic mechanism.

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215.3 **THE FUNCTIONAL PROPERTIES OF THE SEROTONINERGIC CELLS IN LOCUS COERULEUS ON LUMBAR CORD ACTIVITY IN THE CAT.** Y.-Y. Lai, H. K. Strahlendorf, S. J. Fung, and C. D. Barnes. Dept. of Physiology, Texas Tech University, Health Sciences Center, Lubbock, TX 79430.

Previous studies in our laboratory have demonstrated that locus coeruleus (LC) facilitates the monosynaptic reflex (MSR) in both lumbar extensor and flexor mononeuron pools, and this facilitation is partially mediated by catecholamine. Furthermore, we also found both serotonin (5-HT) and norepinephrine (NE) neurons in the LC project to the lumbar spinal cord. This investigation was designated to determine the mechanism of LC effect on the MSRs. Forty-six cats of either sex weighing 2.0-4.0 kg were used. Tracheostomy, cannulation of femoral artery and vein and decerebration were performed under ether anesthesia. A laminectomy was made from the fifth to seventh lumbar segments. The popliteal fossa of the left leg was opened and the medial gastrocnimus and common peroneal nerves were isolated and dissected for stimulation. The L_7 ventral root was dissected near the dura and prepared for recording. A conditioning stimulus was delivered to the LC by a monopolar microelectrode. We found that the LC-induced facilitation of the MSRs is depressed by the intravenous injection of prazosin and/or methysergide at the dose of 20 μg/kg and 1 mg/kg, respectively. This finding suggests that LC-induced facilitation of the MSR is mediated by both NE and 5-HT neurons which innervate the spinal cord. In another experiment, the dorsal and ventral roots from L_6 to S_1 were cut. Intraspinal stimulation was delivered to the L_7 ventral horn. Dorsal and ventral root discharges were recorded from L_7 and ventral roots, respectively. The results demonstrated that the LC consistently facilitates the S and M waves of the ventral root discharge and this facilitation is also depressed by the prazosin and methysergide. (Supported by NIH grant NS 20979 and the Tarbox Parkinson's Disease Institute).

215.4 **LOCUS COERULEUS-INDUCED PRESYNAPTIC FACILITATION OF HINDLIMB AFFERENT ACTIVITY IN CATS.** C.D. Barnes and S.J. Fung. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164-6520.

There has been concordant findings of locus coeruleus (LC)-evoked spinal monosynaptic reflex (MSR) potentiation and LC-induced increase in motoneuron membrane excitability in our laboratory. To explore the concurrent presynaptic action of LC on primary afferent terminals, unanesthetized, decerebrate cats were used with left ventral root (VR) L5 to S1 sectioned intradurally. Placements of the stimulation electrode was achieved by aiming it stereotaxically (P2, L2.5, H-2) to LC with fine adjustments to ensure the presence of VR L7-MSR enhancement by LC stimuli. Histological verification was performed by a combination of Prussian blue reaction and norepinephrine fluorescence histochemical techniques.

LC stimulation (100-300μA, cathodal pulses of 50μsec at 770 Hz, repetition rate of 1 per 5 sec) produced a pure positive dorsal root potential (DRP) recorded ipsilaterally from the caudal-most L6 dorsal rootlets. Conditioning the negative DRPs elicited from individual hindlimb nerve branches with LC stimuli led to a decrease in test DRPs of approximately 10%. While recording peripherally, the antidromic activation of afferent fiber terminals from intraspinal ventral gray stimulus was tested. LC was found to exert a predominant decrease in excitability of afferent terminals of both muscle and cutaneous origins concomitant with an increased motoneuron discharge recorded from the ipsilateral VR L7. These data suggest a presynaptic role of LC on augumenting the afferent transmission through primary afferent hyperpolarization.

Evidence further supports the concept of the coerulospinal induced DRP resulting from inhibition of tonically active interneurons having axoaxonic contacts on primary afferents; functionally, presynaptic disinhibition. The DRP interactions imply a degree of peripheral afferent and coerulospinal convergence on these cells.

Supported by NIH Grant NS20979.

215.5 **POSTSYNAPTIC POTENTIAL AND MEMBRANE CONDUCTANCE CHANGES IN HINDLIMB MOTONEURONS UPON ELECTRICAL STIMULATION OF LOCUS COERULEUS IN CATS.** S.J. Fung and C.D. Barnes. Dept. of Vet. and Comp. Anat., Pharmacol. and Physiol., Washington State University, Pullman, Washington 99164-6520.

Locus coeruleus (LC) stimulation has been shown to lower the rheobase of lumbar motoneurons. This finding has been extended by measuring the postsynaptic potential changes and accompanying changes in membrane conductance of various identified populations of hindlimb motoneurons in unanesthetized decerebrate cats.

Placements of stimulating electrode in the LC were aided by (1) prior recording for LC unit activity, (2) exploring the low threshold sites for potentiation of flexor and extensor monosynaptic reflexes upon LC conditioning, and (3) stereotaxic coordinates (P2 L2.5 H-2) with fine adjustment with reference to anatomical landmarks. Subsequent histological verification of electrode tips were achieved by marking them with +50μA DC current for 17sec with further processing for Prussian blue reaction. In addition histofluorescence techniques were applied to reveal the proximity of stimulation site to noradrenergic LC neurons. LC was stimulated with brief trains of constant cathodal current pulses (50μsec, 1.3msec interpulse interval, 1/sec) while intracellular records were made from identified motoneurons with 3M KCl, 4M K acetate or 2M K citrate-filled micropipettes. Net conductance changes due to LC stimulation were determined by passing single bridge pulses of constant hyperpolarizing current during the peak of postsynaptic potential changes as well as at resting potential.

In 55 cells (spike heights > 58mV) train stimuli (50-500μA) delivered to LC consistently evoked EPSPs with single (37/55) or double peaks (18/55) and with peak amplitudes from 0.2 to 9mV and latency to peak from 6 to 46msec. Single shock to LC-evoked EPSPs of 0.4 to 1mV amplitude and latency to peak from 6 to 27 msec (14 cells). The LC-EPSP was sensitive to alpha-1 blockade by phenoxybenzamine (3mg/kg,i.v.). Membrane conductance during LC-EPSP was estimated to be 11 to 35% higher than at resting membrane potential (9 cells).

Supported by NIH grant NS20979.

215.6 ACTION OF VESTIBULO-SPINAL AND RETICULO-SPINAL FIBERS ON THE PATHWAYS MEDIATING THE PAD OF Ia AND Ib FIBERS IN THE CAT SPINAL CORD. I. Jimenez*, P. Rudomin and M. Solodkin*. Dept. Physiol. & Biophys. CINVESTAV, Mexico 14 D.F. Mexico.

Previous work from this laboratory (1) has shown that cutaneous, rubro-spinal (RS) and cortico-spinal (CS) pathways inhibit the PAD of group Ia fibers produced by stimulation of Ib flexor muscle fibers by acting on the first order interneurons mediating the PAD, and that inhibitory inputs from the brain-stem reticular formation (RF) act on the second (last) order interneurons. Ib fibers are instead depolarized by these segmental and descending inputs. To further characterize the activation patterns of the interneurons mediating the PAD of I fibers, we have now investigated the site of action of vestibulo-spinal (VS) inputs. We used cats anesthetized with pentobarbital (35 mg/kg) supplemented hourly, paralyzed and under artificial respiration. PAD of single I fibers ending in the intermediate nucleus was inferred from changes in their activation threshold, which was automatically determined by means of a digital computer. In 16 out of 36 group I GS fibers classified as Ia because sural (SU) conditioning reversed the PAD generated by group I PBSt volleys (1), stimulation of the VS and of the ipsilateral (i) RF produced PAD which was also inhibited by SU conditioning. In 16 other Ia fibers stimulation of the iRF inhibited the PAD produced by PBSt volleys, as reported previously (1), and only 4 of these fibers showed PAD by VS volleys which was also inhibited by SU and iRF inputs. VS stimulation was without any effect on the other 12 fibers. All Ib fibers tested (n=6) were depolarized by SU, iRF and VS stimulation. Our observations indicate that, unlike the CS and RS fibers which have opposite actions on the interneurons mediating the PAD of Ia and Ib fibers, the VS fibers have excitatory connections with these two sets of interneurons. In the case of the Ia fibers this excitatory action appears to be exerted on the first order interneurons. It is an open question whether stimulation of the reticular formation coactivates two independent descending fiber systems, one inhibiting and the other exciting the interneurons which mediate the PAD of Ia fibers, or whether there is only one descending pathway with segmental excitatory and inhibitory connections. Experiments are now being done to clarify this issue.

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1.- Rudomin et al., J. Neurophysiol. 50 (1983) 743-769.

215.7 THE GROUP IA EPSP IN HUMAN SPINAL SPASTICITY. A Mailis* and P Ashby. Playfair Neuroscience Unit. University of Toronto. Toronto. Canada.

After spinal cord injury many patients develop exaggerated tendon jerks and stretch reflexes, a state known as spasticity. One postulated explanation for this state is that segmental muscle afferents sprout and make new synapses on motoneurons.

Observations were made on 5 normal subjects, 11 patients with incomplete spinal lesions from trauma (3 weeks to 3.5 yrs earlier) and 2 patients with multiple sclerosis. The tibial nerve was stimulated percutaneously at stimulus strengths expressed in terms of the threshold of the alpha motoneuron axons (MT). The action potentials of a single soleus motor unit activated by a voluntary contraction were recorded with an intramuscular needle electrode and extracted using a window discriminator. Post-stimulus time histograms showing the firing probability of the motor unit in relation to the afferent volleys were generated using a lab computer.

The mean interspike intervals of the motor units (and their standard deviations) were not statistically different in the normal and patient groups (Normal (N)=191ms n=6, Spinal (S)=238ms n=22, $p>0.05$). The stimulus strength required to generate a period of increased firing probability (PIF), the threshold for a detectable EPSP was similar in the two groups (N=0.78MT n=8, S=0.79MT n=27, $p>0.7$). The duration of the PIF was similar in the two groups (N=2.03ms n=8, S=2.01ms n=27, $p>0.95$) even for those units considered to have large EPSPs and low background noise. The relative amplitudes of the underlying EPSPs (estimated from the number of extra counts in the PIF per 1000 stimuli) was similar in the two groups (N=66 n=8, S=70 n=27, $p>0.8$).

Thus we detected no changes in the estimated amplitudes or rise times of the group Ia EPSP following spinal lesions in man including those in the subgroup with severe spasticity. There is no support from this study for the hypothesis that sprouting of Ia afferents is responsible for spasticity.

215.8 POTENTIATION OF SYNAPTIC TRANSMISSION FOLLOWING SHORT HIGH FREQUENCY BURSTS AT SINGLE IA/MOTONEURON CONNECTIONS. B.M. Davis, W.F. Collins, III and L.M. Mendell. Dept. Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Stimulation of single Ia fibers with repeated high frequency bursts, (32 shocks at 167Hz every 2 seconds) usually causes the first evoked EPSP in the burst ($EPSP_1$) to be larger than the "standard" EPSP evoked by low frequency (18Hz) stimulation (1). Since each burst (n=128-512) follows the previous one by an interval of 2sec., $EPSP_1$ can be considered to be a measure of the efficacy of transmission 2 seconds after the burst. We suggest that this elevation in $EPSP_1$ which reaches steady state within the first few bursts reflects a process of "potentiation". In the present study, we characterize the time course of "potentiation" between bursts. Single gastrocnemius motoneurons and Ia fibers were simultaneously impaled in anesthetized cats. The Ia fibers were stimulated with high frequency bursts (32 shocks, 167Hz) every two seconds. Additional stimuli [recovery pulses (RP)], were delivered at fixed intervals after each burst to characterize changes occurring between the bursts. The EPSPs were averaged in register. When a single RP was delivered 50 to 100ms after each burst, the resulting EPSP ($EPSP_{RP}$) was generally larger than $EPSP_1$ and always larger than the standard EPSP (by a factor of 1.2-2.6). The greatest relative enlargement of $EPSP_{RP}$ (i.e. with reference to the standard EPSP) was observed at connections with small "standard" EPSPs (<100μV) in which the amplitude of successive EPSPs within the bursts increased (facilitation). In contrast, enhancement of $EPSP_1$ was largest at connections with large standard EPSPs (>100μV). These findings suggest that elevation in $EPSP_1$ amplitude reflects "potentiation" whose time course is relatively slow compared to the facilitation and depression which influence EPSP amplitude during and in the immediate (up to 100ms) aftermath of the burst (i.e. $EPSP_{RP}$). The results also suggest that properties of the synapses differ across connections such that large EPSPs can undergo greater potentiation after the burst as well as more depression during the burst (1) than small EPSPs. Such activity and time-dependent changes associated with short bursts of firing emphasize the necessity to consider the group Ia fiber firing pattern in assessing efficacy at individual Ia/motoneuron connections during different tasks which can involve bursts of high frequency firing. Support by NIH Grants NS07319(BMD), NS06407 & NS20264 (WFC) & NS14899 & NS16996(LMM).

(1) Collins, W.F.III, Honig, M.G. and Mendell, L.M. Neurosci. Abstr. 9, 1983.

215.9 VARIATION OF SPINAL MOTONEURON EPSP AMPLITUDE DURING FREQUENCY MODULATED STIMULATION OF SINGLE IA AFFERENT FIBERS, W.F. Collins, III, B.M. Davis and L.M. Mendell. Dept. Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

Stimulation of single Ia afferents with high frequency bursts can result in considerable (3 fold) modification of motoneuron EPSP amplitude both during and after (up to 30sec) the stimulation (B.M. Davis et al. this volume). In the present study we examine the modulation of EPSP amplitude during frequency modulated group Ia impulse activity. The discharge pattern (52 impulses with interspike intervals ranging from 5.2 to 55.9ms) was that of a single vastus medialis spindle recorded during a period of isometric contraction in a walking cat (courtesy of Dr. G.E. Loeb). Single gastrocnemius motoneurons and Ia afferents were simultaneously impaled in Nembutal anesthetized cats. The burst was presented 64-256 times with an 800ms interburst interval which corresponded to the rate of stepping. The motoneuron EPSPs were averaged in register so that the amplitude modulation of the EPSPs during the burst was clearly visible. The pattern of modulation differed from connection to connection. In some cases, particularly in low rheobase motoneurons, EPSPs produced by the high frequency portion of the burst (stimuli 23-27) were diminished in amplitude. In others, little or no amplitude modulation occurred. These differences in modulation were of sufficient magnitude to reverse the rank order of the different connections as determined by EPSP amplitude. At a few connections, particularly on motoneurons with large rheobase, an increase in amplitude was observed during the high frequency portion of the burst. We also noted that the initial EPSP in the burst was the largest EPSP, and that in most cases it was larger than the "standard" EPSP generated by low frequency (18Hz) stimulation. Amplitude modulation within these bursts ($EPSP_1/EPSP_{23-27}$) could be as high as a factor of 3. These findings emphasize that EPSP amplitude is not fixed but varies systematically during frequency modulated activity in the presynaptic fiber. The finding that EPSP amplitude modulation correlates with motoneuron rheobase suggests that transmission of Ia fiber information to α-motoneurons may differ systematically according to the different types of motor units (S,FR,FF) they supply since, in general, progression from S to FR to FF is associated with an increase in motoneuron rheobase (Fleshman et al. J. Neurophysiol. 46, 1981). Support by NIH NS06407 & NS20264(WFC), NS07319 (BMD) and NS14899 and NS16996(LMM).

215.10 EXCITABILITY OF DISTAL FORELIMB MOTONEURONS. B.R. Botterman and T.C. Cope. Dept. of Cell Biology, Univ. of Texas Hlth. Sci. Ctr., Dallas TX 75235

Recently obtained knowledge about the mechanical properties of distal forelimb motor units (Botterman et al., Soc. Neurosci. Abstr. 8:330, 1982) raised questions about properties of their motoneurons (MN). In particular, we wanted to determine if mechanical differences in these motor units relative to those of the hindlimb might be paralleled by differences in MN repetitive firing and other discharge properties.

Intracellular recordings were made from median and ulnar MNs in barbiturate-anesthesized cats. In 15 MNs exhibiting action potential amplitudes >65mv, depolarizing current pulses were injected for durations appropriate to study after-hyperpolarization (AHP; 0.1ms), rheobasic current (50ms) and repetitive firing (2.5s). AHP half-decay time ranged from 12-50ms, while amplitude varied between 1.4-6.2mV. Rheobasic current ranged between 1-16nA. These values fall within previously reported ranges for hindlimb MNs.

Repetitive firing of 11 MNs was evaluated over at least 4 current strengths. The minimum steady state firing rate, measured 1s after the onset of current injection, ranged between 12-31pps, and was directly related ($P<0.01$) to rheobasic current. Current threshold for repetitive firing was about 2 times greater than rheobasic current. The steady state frequency/current slope in the primary range varied between 0.77-2.21pps/nA. The maximum steady state firing rate in the primary range was between 26-83pps. The greatest instantaneous firing rates, 333-454pps, were observed for the first interspike interval in the secondary range, and these values were inversely related ($P<0.01$) to AHP amplitude.

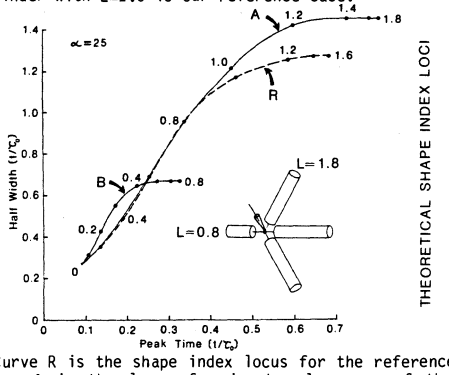
These preliminary observations indicate that the intrinsic discharge properties of distal forelimb MNs closely resemble those of hindlimb MNs. This similarity further suggests that differences in speed-related mechanical properties between hindlimb and distal forelimb motor units is not reflected in their respective MN discharge properties measured in this study. Supported by NIH grant NS 17683.

215.11 CONSEQUENCES OF THE FUNCTIONAL VARIATION OF MOTONEURONE POSTSYNAPTIC PROPERTIES ON EPSP SHAPE INDICES. B. Gustafsson* and M.J. Pinter. Dept. of Physiol., Univ. of Göteborg, Box 33031, S-400 33 Göteborg, Sweden.

Among cat triceps surae motoneurones (MN), the shapes of composite Ia EPSPs differ such that both rise-times and half-widths are longest among MNs projecting to slow twitch muscle units. Since previous evidence indicated that no substantial differences existed among MNs in either specific membrane properties or dendritic geometry, these shape variations have been attributed to differences in the positioning of Ia synapses along the dendritic tree. More recent evidence indicates, however, that there exists a substantial variation in specific membrane resistivity and dendritic geometry among MNs. We were interested in exploring the consequences of this on EPSP shape indices. Ten-compartment equivalent cylinder models were assembled using average properties obtained from 2 MN groups classified according to rheobase current and input conductance. These groups are referred to as fast and slow since they possess average rheobase similar to types S and FF MNs. A conductance transient of the form $\alpha t \exp(1-\alpha t)$ and time-to-peak of 150 μsec ($1/\alpha$) was injected into individual compartments and the shape indices of the resulting 'somatic' EPSPs were calculated. For each injection site, the EPSP half-widths, times-to-peak and 10-90 % rise-times were longer in the slow model, and the differences became more exaggerated at successively more distal injection sites. When the current was distributed uniformly among compartments 3 to 6, the resulting shape indices for the slow model were quite similar to the apparent average values observed for type S composite Ia EPSPs. For the fast model, the shape indices fell within the lower part of the range observed for type F composite EPSPs. The results of these calculations suggest that composite EPSP shape-index differences may arise from systematic differences in MN postsynaptic properties rather than differences in dendritic location of synaptic input.

215.12 EPSP SHAPE INDICES WHEN DENDRITIC TREES HAVE UNEQUAL LENGTH. Idan Segev* and Wilfrid Rall, Mathematical Research Branch, NIADDK, NIH, Bethesda, MD 20205.

A number of studies use EPSP shape indices to estimate the cable distance of localized dendritic synaptic input from the neuron soma. These estimates are usually based upon shape index loci (plots of half width versus peak time for several input locations) computed for a model which represents all dendritic trees as equivalent to cylinders of the same electrotonic length, L. Here we address the complication that results when several dendritic trees belonging to one neuron have different L values. We have an analytical solution for this case, which provides the complete EPSP shape produced by local injection of current proportional to T exp ($-\alpha$T). We illustrate our results with a quadripolar model having one short tree with L=0.8, three long trees with L=1.8, and with a very small soma. In this case the decay time constants and coefficients yield an L value estimate for the system (L_{peel}) of 1.6 (Segev and Rall, Neurosci. Abstr., 1983); thus a single cylinder with L=1.6 is our reference case.



Curve R is the shape index locus for the reference case. Curve A is the locus for inputs along any of the three long trees; this locus diverges from the reference case only for distal input locations. Curve B is the locus for inputs along the short tree; near its midpoint, curve B crosses the reference curve; its proximal half is steeper and its distal half is flatter and lower.

215.13 COMPUTER SIMULATION OF COMPOSITE EPSPs BASED ON THE MORPHOLOGY OF α-MOTONEURONS AND GROUP Ia AFFERENTS. R. E. Burke, J. W. Fleshman and I. Segev*. Lab. Neural Control, NINCDS, and Math. Res. Br., NIADDK, NIH, Bethesda, MD.

Last year, we reported (Neurosci. Abstr. 9:341) that the input resistance (R_N), membrane time constant, and electrotonic length of six HRP-labeled, type-identified medial gastrocnemius (MG) α-motoneurons could be reconciled with their morphology when they were simulated in computer models with non-uniform specific membrane resistivity (R_m). R_m could either rise abruptly from a low value at the soma to a higher, constant value in the dendrites (step model) or monotonically increase as a function of distance from the soma (sigmoidal model). In both cases, specific membrane capacitance was 1 $\mu F/cm^2$. In the present study, the same motoneuron models were used to examine the EPSPs produced by model synapses spatially distributed in accord with HRP studies of group Ia contacts onto triceps surae motoneurons (Glenn et al., Neurosci. Abstr. 8:995, 1982). A total of 300 synapses was activated (60 MG Ia afferents times 10 boutons per afferent times 0.5 probability of transmitter release), each with a peak conductance of 5 nS at 200 μs (Finkel and Redman, J. Physiol. 342:615, 1983). Synaptic conductance onsets were distributed in time in accord with conduction latency differences among cat group Ia afferents (Luscher et al., J. Neurophysiol. 42:1146, 1979). Simulations were carried out using the general purpose network analysis program "SPICE" running on a VAX-11/750 computer. The main results to date are:

1. For a given motoneuron and spatial distribution of synapses, the composite EPSPs generated in the two R_m models were virtually identical in amplitude and shape.
2. Calculated EPSPs were similar in amplitude and shape to group Ia EPSPs observed experimentally in MG motoneurons of the appropriate motor unit type.
3. Peak composite EPSP amplitudes were highly correlated with the R_N of the model neurons.
4. The inverse correlation between EPSP amplitude and total cell membrane area (and consequent direct correlation with the density of active synapses) was less precise because a very high soma conductance had to be used to match electrophysiological properties with morphology in one motoneuron.

215.14 COMPUTER SIMULATION OF SINGLE FIBER EPSPs BASED ON THE MORPHOLOGY OF α-MOTONEURONS AND GROUP Ia AFFERENTS. J. W. Fleshman, I. Segev* and R. E. Burke. Lab. Neural Control, NINCDS, and Math. Res. Br., NIADDK, NIH, Bethesda, MD.

Previous work in this laboratory (Glenn et al., Neurosci. Abstr. 8:995, 1982) described the spatial organization of synaptic contacts made by individual triceps surae group Ia afferents onto homonymous and heteronymous α-motoneurons. In the present work, we used these anatomical data to simulate the synaptic potentials that would be generated by single boutons (SB-EPSPs) and by single group Ia fibers (SF-EPSPs; 3-32 boutons simultaneously activated) in electrophysiologically and morphologically characterized medial gastrocnemius (MG) α-motoneurons. The local amplitude of SB-EPSPs in different cells ranged from 75-275 μV for a somatic bouton, to 20-30 mV for a bouton located on a distal dendrite. SF-EPSPs differed in shape and amplitude depending on the number and postsynaptic spatial distribution of boutons from individual afferents. For a given afferent, SF-EPSPs were similar in the step versus sigmoidal R_m models (see companion abstract). For each cell, the shape indices of the simulated SF-EPSPs were compared to two reference curves: 1) shape index loci computed for the conventional representation of a motoneuron as a single cylinder, with uniform R_m and L_{peel} matching the model neuron (see Segev and Rall, this meeting); 2) an empirical shape index curve obtained by activating synapses in concentric rings at regular intervals of electrotonic distance, using the cell models with non-uniform R_m. Our main results to date are:

1. Even when the boutons of a single fiber are electrotonically widely dispersed, the rising and falling phases of the EPSP recorded at the soma are usually without inflection.
2. The shape indices of about 1/3 of the simulated SF-EPSPs fell near reference curve 2, as if originating from restricted electrotonic loci. In the others, the spatial dispersion of boutons resulted in half-widths that were too long for the corresponding peak times.
3. The mean DC electrotonic distance from the soma to the boutons of a single afferent was consistently underestimated when reference curve 1 was used. This result emphasizes that accurate estimates of the electrotonic distribution of synaptic inputs using shape index plots require accurate electrical models of the postsynaptic cell.

215.15 IS STEM DENDRITIC DIAMETER A GOOD ESTIMATOR OF TOTAL DENDRITIC SURFACE AREA OF A SPINAL MOTONEURON AS REVEALED BY INTRACELLULAR INJECTION OF HRP? W.E. Cameron, D.B. Averill and A.J. Berger. Dept. of Physiol. & Biophysics, Univ. of Washington, Seattle, WA 98195.

The dendrites of a spinal motoneuron constitute the major surface area (SA) available for receipt of synaptic inputs. It has been proposed for motoneurons of the lumbosacral cord that the diameter of the stem dendrites is an accurate estimator of total dendritic SA (Ulfhake & Kellerth, J. Comp. Neurol. 202:571, 1981). We undertook the present analysis to test the relationship between total dendritic SA and stem dendritic diameter for a cervical motoneuronal population, the phrenic motoneurons (PMs).

All 37 dendrites produced by four PMs were analyzed in detail following intracellular injection of horseradish peroxidase (HRP). The cells were reconstructed from horizontal sections. Somal dimensions and dendritic lengths were quantified from the reconstructions using a digitizing tablet. Dendritic diameter was measured directly using an eyepiece micrometer. The SA of each dendritic segment was calculated using the length and mean diameter of the segment. No correction was made for shrinkage due to histological processing.

The stem dendritic diameter of a PM was positively correlated with its combined dendritic length, number of terminal branches, dendritic SA and volume. The accuracy of stem diameter as a predictor of total SA was assessed by comparing the values calculated from the power equations derived from the relationship above with those derived from direct measurements. The values differed by greater than 10% for two of four cells analyzed. The 37 dendrites studied could be subdivided into five groups based upon the initial projection of their stem dendrites. An analysis of variance revealed a significant F-ratio ($P<.05$) for several geometric parameters measured for the different groups indicating that the PM dendrites are not a homogeneous population. Comparisons between groups for these quantities revealed significant differences ($P<.05$) using a modified t-statistic. We conclude that the stem dendritic diameter may not be a good estimator of total dendritic SA for this cervical motoneuronal population due to heterogeneity of the dendrites. (Supported by USPHS grant NS 14857, NRSAs HL 06474 & 06233 and an MDA Postdoctoral Fellowship)

215.16 REPONSES OF POPULATIONS OF DSCT NEURONS TO NATURAL STIMULATION. C. E. Osborn and R. E. Poppele, Lab. of Neurophysiol., Univ. of Minnesota, Minneapolis, MN 55455

Single unit impulse activity from dorsal spinocerebellar tract (DSCT) neurons of barbiturate anesthetized cats was recorded in the presence of various natural stimuli. Brief stretches or twitch contractions of the gastrocnemius soleus (GS) or brief flexions of the intact ankle were randomly presented in time. For each cell, the change in firing probability with respect to its background firing rate was calculated from post stimulus time histograms. Excitability changes in a population of DSCT cells were then estimated from an average of normalized responses in a large number of single units subjected to the same stimulus. The estimated population response revealed three major groups. For at least 40 ms following the stimulus, one group was only excited, another only inhibited and a third was first excited and then inhibited (only a few, <5% were first inhibited and then excited). Preliminary results are summarized below.

The three response types were about uniformly distributed within any sample of DSCT neurons and most of the cells responded to each stimulus presented (92% responded on average). Furthermore, the distribution of response types for a given stimulus was not dependent on the cells' response to other stimuli. For example, cells that were only excited by muscle contraction responded to stretch with the same response distribution as other sample groups. The results imply that proprioceptive inputs are each distributed to most of the units of the DSCT in a seemingly random manner. Single unit behavior is therefore likely to reflect a considerable convergence from sensory receptors.

Supported by NIH grant NS 07147.

STIMULUS	# CELLS	% EXC	% INH	% E-I
GS STRETCH	92	31%	29%	36%
GS CONTRACT	126	32%	25%	37%
ANKLE FLEX	160	38%	29%	33%
CELLS EXCITED BY GS CONTRACTION				
GS STRETCH	40	40%	27%	33%
CELLS INHIBITED BY GS CONTRACTION				
GS STRETCH	30	30%	39%	31%
CELLS EXCITED BY GS STRETCH				
GS CONTRACT	27	41%	33%	26%

215.17 SENSORY AFFERENTS TO RED NUCLEUS THROUGH COLLATERALS OF FIBRES RUNNING IN THE DORSAL COLUMNS OF THE SPINAL CORD. E. SYBIRSKA+ and Y. PADEL. Lab. of General Neurophysiology, CNRS , INP , F13277 Marseilles , France.

Several studies performed on awake cats have shown that rubrospinal cells receive an exteroceptive input : natural stimulation to the skin could activate the cells or inhibit their tonic activity. Intracellular recordings in acute experiments indicate that electrical stimulation to the paw induces in rubrospinal cells a complex response composed of a mixture of EPSPs and IPSPs. Destruction of the whole cerebellum and of the frontal part of the cerebral cortex (which are the two structures known to send monosynaptic projections to the red nucleus) does not result in the disappearance of the sensory responses in rubrospinal cells.

In the same preparation, stimulation of the dorsal columns with low intensity currents ($\leqslant 30\mu A$) induces the same composite response in rubrospinal cells. With a latency around 5 msec after stimulation it appears a long lasting EPSP often curtailed by a profound IPSP. This indicates that the fibres responsible for the responses in rubrospinal cells, or their collaterals, are running in the dorsal columns of the spinal cord.

In subsequent experiments, it was shown that a section of the dorsal columns of the spinal cord does not abolish the responses induced in rubrospinal cells by natural stimulation of the paw. This fact indicates that an other pathway than the dorsal columns could be involved. Furthermore, low current surface stimulation of the dorsal columns, caudally to their section still induces responses of the same kind as before the section. In addition, stimulation of the dorsal columns rostrally to the section also evokes the same responses in rubrospinal cells. The amplitudesof the responses induced from dorsal columns stimulation, caudally or rostrally to the section, are dependant of the level of the section. It was observed in some cells that, if the section is in the rostralmost segments of the spinal cord, the response evoked from the electrode placed caudally to the lesion is larger than from the rostral electrode and vice versa when the section is in a more caudal segment.

It could be concluded from the above experiments that the primary afferents give off collaterals to the dorsal columns and in addition to an other pathway in the spinal cord. This other pathway conducts the somatic messages to the rubrospinal cells, through an extracerebral and extracerebellar route.

215.18 Cat Red Nucleus Projects to Digit Extensor Motorneurons M.L. McCurdy*, D.I. Hansma, J.C. Houk and A.R. Gibson
Northwestern Medical School, Chicago, IL 60611

Previous investigators using autoradiographic (Holstege & Kuypers, Prog. Brain Res., '82) and degeneration (e.g. Nyberg-Hansen & Brodal, J. Anat, '64) techniques have demonstrated that the rubrospinal pathway terminates in the intermediate spinal gray (Rexed's Laminae V-VII) throughout the length of the cord. We have examined the rubrospinal terminations using anterograde transport of WGA-HRP. As seen with the other techniques, the vast majority of terminals are in intermediate gray areas. However, at spinal level C8 a focus of terminals can be seen within a pool of laterally placed motorneurons. The position of the motorneuron pool suggests that these neurons innervate distal extensor musculature. To identify the motorneuron pool, we injected extensor digitorum communis and extensor digitorum lateralis with multiple injections of 20% HRP mixed with 5% NP-40. Additionally, the red nucleus homolateral to the injected muscles was injected with .014ul of 1% WGA-HRP. In this way, the positions of retrogradely labeled motorneurons and anterogradely labeled terminals could be compared between sides of the same spinal section. The location of the labeled neurons and terminals corresponded precisely, and there was no focus of terminal label around other motorneuron pools. Thus, the cat magnocellular red nuclues appears to have selective monosynaptic terminations on digit motorneurons.

Although the motorneuronal terminations are slight in comparison with the interneuronal terminations, their distribution is interesting in light of behavioral findings. Sybirska & Gorska (Acta. Neurobiol. Exp., '80) have reported that cats with rubrospinal lesions mainly suffer an impairment in digit control. Chronic recording studies in monkeys (Kennedy, Gibson & Houk, Soc. Neurosci Abst., '83; Kohlerman, Gibson & Houk, Science, '82) have found that most magnocellular red nucleus neurons are active during digit use, and lesion studies of the monkey (Lawrence & Kuypers, Brain, '68) show that the red nucleus is important in hand control. It would seem that the innervation of digit extensor motorneurons by the red nucleus reflects the prominent role which the red nucleus plays in movements requiring digit use.

215.19 PROJECTION OF MUSCLE SPINDLE AFFERENTS TO DORSAL NECK MUSCLE MOTONEURONS REVEALED BY SPIKE TRIGGERED AVERAGING. S.A. Keirstead and P.K. Rose. Department of Physiology, Queen's University, Kingston, Ontario. K7L 3N6

Recent anatomical and physiological studies suggest that the segmental connections of dorsal neck muscle spindles may differ from those of hindlimb muscle spindles. For example, monosynaptic composite EPSP's recorded in dorsal neck muscle motoneurons in response to stimulation of muscle spindle afferents are unusually small (350-3100 uV, Brink et al. J. Neurophysiol. 46: 496, 1981), particularly in light of the large number of muscle spindles in dorsal neck muscles (Richmond and Abrahams, J. Neurophysiol. 38:1322, 1975). This result may be a consequence of individual muscle spindles projecting to only a small percentage of dorsal neck muscle motoneurons. Alternatively, the percent connectivity may be high, but the size of the unitary EPSP's could be small. The present experiments were designed to examine these possibilities.

All experiments were performed on paralyzed, anaesthetized (sodium pentobarbital) cats. Muscle spindle afferents with primary-like activity were recorded in the dorsal funiculus in C1 or C2 and their activity was used to trigger and average (1024-2048 samples) intracellular recordings obtained from dorsal neck muscle motoneurons. Unitary EPSP's were observed in only 10% of the motoneurons examined (9/89). The amplitude of unitary EPSP's was, on average, 50 uv (range 17 - 85 uv). Preliminary analysis of the rise times and half widths of these unitary EPSP's indicated that the majority of synaptic contacts on dorsal neck muscle motoneurons were on the proximal dendritic tree. Motoneurons in which EPSP's were recorded were not evenly distributed within the motoneuron nucleus, but rather, tended to lie together in "colonies".

These results suggest that the primary reason for the small amplitude of composite monosynaptic EPSP's is the low percent connectivity of individual dorsal neck muscle spindle afferents. This unusual arrangement is consistent with the wide intercollateral spacing (average of 1 collateral/4 mm of axon) and restricted longitudinal extent of the ventral horn termination zone (usually less than 1000 um) of dorsal neck muscle spindle afferents. (Supported by the MRC of Canada).

SPINAL CORD AND BRAINSTEM III

216.1 RESPIRATORY ACTIVITY IN A PERFUSED GUINEA PIG BRAIN/SPINAL CORD PREPARATION G.B. Richerson* and P.A. Getting. Dept. of Physiol. Biophys., Univ. of Iowa, Iowa City, IA 52242.

Arterial perfusion of the central nervous system has a number of advantages. It allows control of arterial ion concentrations, metabolic factors, and drug application. The absence of arterial pressure pulsations and lung movements gives sufficient mechanical stability to allow long-term intracellular recordings of CNS neurons. The isolated, perfused rat brain has already been used to study amino acid uptake, neurotransmitter turnover, and drug actions. An isolated, perfused guinea pig cerebellum preparation has also been used to study intracellular activity in Purkinje cells.

We have developed a perfused guinea pig brain preparation with the spinal cord intact to the third vertebra. Animals were anesthetized with Nembutal (40 mg/kg IM) and Innovar-Vet (0.35 cc/kg IP), and given a thoracotomy. The left ventricle of the heart was cannulated with a needle and perfused using a perfluorocarbon in Ringer's with Urethane (13 mM). The descending aorta, carotid, and subclavian arteries were then ligated. Latex injections of the remaining arterial tree revealed that the cervical spinal cord and the entire brain, including the cerebral cortex, were perfused. This is due to the absence of any internal carotid artery in the guinea pig. Peripheral nerve activity was recorded using cuff electrodes.

Viability of perfused CNS preparations has been monitored using a number of criteria, including EEG, simple reflex responses (e.g. corneal reflex), intracellular activity of single neurons, glucose metabolism, oxygen uptake and structural changes. Normal function of the central nervous system, however, would require maintenance of complex synaptic interactions between large numbers of different neurons. Using the perfused guinea pig brain/spinal cord preparation, we have recorded rhythmic burst activity from the phrenic nerve indicative of "fictive" respiration. This suggests that integrated neural function was maintained in the perfused brain/spinal cord preparation. Fictive respiration was recorded for as long as two hours. Because the venous effluent was not recycled, the duration of recording was limited solely by the volume of perfusate. This preparation should allow stable intracellular recordings from central neurons of the respiratory system and may be useful for studying neurons throughout the CNS. (Supported by NS15350).

216.2 EMG Correlates of Apomorphine-Induced Rhythmic Jaw Movements in the Guinea Pig. R.W. Lambert*, L.J. Goldberg and S.H. Chandler (SPON: W.R. Salafia). Depts. of Oral Biology, Kinesiology, and Anatomy, UCLA, LA., CA 90024.

Persistent gnawing is a behavioral stereotypy obtained in many species in the awake, freely behaving state following the administration of apomorphine (APO). Similarly, we have observed rhythmic jaw movements (RJMs) in the ketamine anesthetized guinea pig following APO administration. The purpose of the present study was to describe the jaw movement trajectories during RJMs produced by APO along with the underlying muscle coordination patterns.

EMG electrodes were implanted under ketamine anesthesia (100 mg/kg) in the anterior digastric (DIG), lateral pterygoid (LP), medial pterygoid (MP) and deep masseter (MASS) muscles bilaterally in 8 guinea pigs (500-900 g). A small light source was affixed to the inferior surface of the mandible and a photoelectric position sensor was used to detect vertical and horizontal excursions of the jaw.

Approximately 4-6 min. following APO administration (2 mg/kg i.v.), RJMs began to appear and continued virtually without pause for 40-60 min. The opening and closing phases of individual RJM cycles did not demonstrate any lateral movement. The onset of coincident bilateral activity (≈160 ms burst duration) in both the DIG and LP muscles preceded the onset of the opening movement by 7-10 ms. The MP muscles were also active bilaterally during opening, but weakly. The closing phase began after the cessation of activity in the DIG, LP and MP muscles, and was associated with activity in the MASS muscles bilaterally (burst duration ≈150 ms).

Occasionally, a second form of RJM, possessing a similar cycle time (250-400 ms), was evident following APO administration. Similar to RJMs spontaneously occurring in the absence of APO, these cycles were characterized by relatively short burst durations (≈70 ms) and the frequent appearance of bilateral asymmetries in the amplitudes of both the DIG and LP muscles during opening. Closing followed a lateral trajectory and began 7-10 ms following the onset of a large amplitude burst in the MP muscle contralateral to the movement. Activity in the MASS muscles was absent during these cycles. The evidence suggests that apomorphine activates a dopamine-sensitive pathway resulting in a form of RJM which is distinguishable from RJMs observed in other situations especially in terms of the absence of a lateral component of jaw movement.

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216.3 Role of the Medial and Lateral Pterygoid Muscles in Producing the Lateral Components of Spontaneous Rhythmic Jaw Movements in the Guinea Pig. L.J.Goldberg, R.W. Lambert* and S. H. Chandler. Depts. of Oral Biology, Kinesiology and Anatomy and Brain Research Institute, UCLA, LA., CA 90024.

We have previously shown that during rhythmic jaw movements in the anesthetized guinea pig there is a coordination of activity between jaw opening and jaw closing motoneurons (Goldberg et al., J. Neurophysiol., 48:110, 1982). In the present experiments we extended the study to include lateral jaw movements.

In albino guinea pigs (500-900 g) anesthetized with ketamine HCl (100 mg/kg), EMG recordings were obtained from the digastric (DIG), deep masseter (MASS), medial and lateral pterygoid (MP and LP) muscles bilaterally. Spontaneous rhythmic jaw movements (SRJMs) were monitored with the use of a photoelectric positon sensor placed in front of a small tungsten light source fixed to the inferior surface of the mandible. During each SRJM cycle two invariant horizontal movements were observed; the first was associated with jaw opening and the second with jaw closing. ≈20 ms after the onset of the bilateral DIG EMG activity which initiated each cycle by producing a jaw opening movement, either the left or right LP muscle became active. The contraction of the DIG and LP muscles produced a combined opening and lateral movement of the jaw. This phase of the cycle was terminated by the end of the ≈60 ms burst of EMG activity in the DIG and LP muscles. The second phase of movement then began. It was characterized by a jaw closing movement combined with a lateral jaw movement in the same direction as that initiated during the opening phase. This combined closing and lateral jaw movement was initiated by a burst of activity (burst duration ≈40 ms) in the MP muscle contralateral to the direction of the movement. Low levels of EMG activity in the MASS muscle were occasionally observed during the closing phase of the cycle; however, the onset of this activity occurred after the initiation of the closing movement.

We have demonstrated that the LP and MP muscles are critical for the coordination of vertical and lateral jaw movements during SRJMs. This coordination is accomplished by the sequential activation of first the LP and then the MP muscle on the side contralateral to that to which the movement is to occur. The lateral movement observed during jaw opening is produced by the LP muscle, and the lateral movement that occurs during closing is produced by the MP muscle.

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216.4 A Coupled Oscillator Model of Spontaneous Rhythmic Jaw Movements in the Guinea Pig. A. Garfinkel*, L.J. Goldberg, R.W. Lambert* and S.H. Chandler (SPON: S.H. Chandler). Crump Institute for Medical Engineering, Depts. of Kinesiology, Oral Biology and Anatomy, UCLA, L.A., CA 90024.

Spontaneous rhythmic jaw movements (SRJMs) in anesthetized guinea pigs are complex movements requiring coordinated activities in a number of dimensions. We studied the forms of mandibular movement in the X-Y dimensions as a possible clue to underlying neural mechanisms.

Phase plane analyses show complex phase cycles: i.e., sequences of definite stages. The vertical movement has four phases: an accelerated opening, an accelerated closing, a pause, then an unaccelerated opening "drift." The horizontal movement consists of two subcycles, each one having a phase cycle consisting of a lateral movement (small acceleration) followed by a second lateral movement consisting of a large acceleration continuing in the same direction, followed by a large acceleration back to the midline, and ending in a pause. The phase decomposition enables us to state several basic invariants. Variations in overall cycle times are not accompanied by variations in the lengths of the active (accelerated) phases; the variations are taken up by variation in the pause phases. Very strong coordinations were found to exist between specific phases of the horizontal and vertical movements. The fast opening phase is synchronized to the small acceleration lateral movement, the closing phase is synchronized to the large acceleration lateral movement, and the pause phase of the horizontal is synchronized with the pause and slow opening phases of the vertical movement.

The phase cycle of the horizontal oscillation suggests that the horizontal oscillator is itself composed of two oscillators coupled by reciprocal inhibition, one for movement to and on the left, and one for movement to and on the right. This is required by the fact that each lateral cycle acts as an independent unit synchronized to one cycle of the vertical movement. The overall movement may repeat on the same side several times or "flip" to the other side.

Our preliminary model of SRJMs consists of a set of coupled oscillators: a pair of horizontal oscillators linked to each other by inhibitory coupling, with this pair then linked by synchronizing (excitatory) couplings to the vertical oscillator, with additional synchronizations of horizontal and vertical phases.

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216.5 CYCLIC VOLUNTARY JAW MOVEMENTS: KINEMATIC AND EMG PATTERNS. R. Stiles and S. Wallace*. Depts. of Physiology & Biophysics, and Orthodontics, Univ. Tenn. Center for the Health Sciences, Memphis, TN 38163

Voluntary cyclic movements of a body part occur during mastication, speech, locomotion, writing, etc. Such "periodic" movements may normally involve relatively complex kinematic and muscle activation patterns. For this study, the waveform and timing of agonist-antagonist muscle activation, and resulting acceleration, velocity, and displacement waveforms were analyzed for cyclic voluntary movements of the mandible.

Mandibular acceleration and surface EMGs from masseter, temporalis and mylohyoid muscles were recorded during voluntary, self-paced, cyclic opening-closing of the jaw at frequencies between 1 and 2 Hz. Records were also obtained during maximum effort, rapid jaw opening. All data were obtained from normal adult human subjects. Jaw acceleration in the vertical plane was detected by an AVR-250 accelerometer fastened onto the platform of a device which was 1) attached to a wire brace mounted on the lower teeth, and 2) stabilized at the chin. Velocity and displacement records were obtained by digital integration. EMGs were digitized, rectified and smoothed. Acceleration records of voluntary jaw movements showed little evidence of jaw tremor oscillations, and therefore were analyzed in the time, as well as the frequency, domain.

Results indicate that, for these rapid movements, depressor and elevator muscle activity is "turned on" and "off" at or near the extremes of jaw position during cyclic movements. This reciprocal activation pattern, together with the braking effect of muscle-joint mechanics, resulted in two acceleration-deceleration pulses of the jaw during each position cycle. This acceleration pattern resulted in a single biphasic pulse of velocity and a single, nearly sinusoidal cycle of position. This kinematic pattern is similar to that seen during rapid movement of other body parts, and approximates that calculated for a "maximum-effort, minimumtime optimum bang-bang servo" model for control of movement of a mass (Smith, BME-9:125-128, 1962).We propose that a biphasic pattern of acceleration results because muscles pull but do not push, and therefore are "turned on" near the extremes of position during rapid cyclic movements. We suggest that this characteristic of muscle places an important constraint on the pattern of central control of rapid cyclic movements, and on the kinematic pattern.

216.6 THE SPONTANEOUS IPSPS INVOLVED IN MOTONEURON INHIBITION DURING ACTIVE SLEEP ARE BLOCKED BY STRYCHNINE. F.R. Morales, A. Baranyi*, P.J. Soja*, and M.H. Chase. Depts. of Physiology and Anatomy and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

During active sleep (AS), discrete spontaneous inhibitory synaptic potentials impinge on spinal cord alpha motoneurons (Mns). The waveform characteristics of these potentials have been previously described (Morales and Chase, Exp. Neurol. 78:471-476, 1982; Morales, et al., Soc. for Neurosci. Abstr. 9:735, 1983). The present work is part of an ongoing project designed to determine the pharmacological antagonists of this new type of postsynaptic inhibitory process. Accordingly, antagonists of putative inhibitory neurotransmitters (strychnine [STY] and picrotoxin [PTX]) were iontophoretically injected extracellularly onto lumbar Mns in the chronic cat.

Iontophoretic applications of STY and PTX were performed during quiet sleep preceding and during the transition to AS. The concentration of STY in the drug barrels ranged from 5 to 10 mM. Iontophoretic currents ranged from 30 to 200 nAmps and lasted from 30 sec to 2 min. The overall effect of STY consisted of suppression, and occasionally complete blockade, of the discrete AS-specific IPSPs (18 Mns). PTX (saturated solution in 165 mM NaCl), which was injected with a current ranging from 75 to 250 nAmps for 30 sec to 2 min (10 Mns), did not modify either the frequency or the waveform characteristics of these AS-specific spontaneous IPSPs. In control experiments, STY and PTX blocked the effects of iontophoretically injected glycine and GABA, respectively, indicating that the antagonist drugs were being released from the micropipettes. To the extent that strychnine is an effective antagonist of glycine and glycine-like substances, our working hypothesis is that glycine is the neurotransmitter utilized by the inhibitory interneurons promoting these AS-specific IPSPs.

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216.7 THE IPSPS INDUCED IN CAT LUMBAR MOTONEURONS DURING ACTIVE SLEEP BY STIMULATION OF THE MEDULLARY RETICULAR FORMATION ARE STRYCHNINE-SENSITIVE. P.J. Soja*, F.R. Morales, D.R. Navarrete* and M.H. Chase. Depts. of Physiology and Anatomy and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Electrical stimulation of the nucleus gigantocellularis (NGC) elicits a chloride sensitive, inhibitory potential (IPSP) in feline lumbar motoneurons during active sleep (AS) (Soc. Neurosci. Abstr. 9:664, 1983). The present experiments were performed in an attempt to identify the inhibitory substance(s) mediating this AS-related IPSP activity.

Seven cats were prepared for chronic intracellular recording from lumbar motoneurons during sleep and wakefulness as previously described (Physiol. Behav. 22:355, 1981). Composite 4-barrel "parallel" glass microelectrodes permitted intracellular recordings from antidromically identified lumbar motoneurons and simultaneous extracellular drug iontophoresis. A recording barrel (filled with 2M K^+ citrate) protruded 40--100 microns beyond the affixed drug barrels (tip diameter 4--7 microns). The extracellularly directed barrels contained the following solutions: glycine (GLY, 1.5 M, pH 3.6), gamma-aminobutyric acid (GABA, 1.5 M, pH 4.2), strychnine nitrate (STY, 5--10 mM in 165 mM NaCl), picrotoxin (PTX, saturated in 165 mM NaCl), or 165 mM NaCl as a control solution and for automatic current control.

Prior to the transition into AS, iontophoretic GLY and GABA (30--100 nAmps) hyperpolarized lumbar motoneurons and blocked spike potentials elicited by intrasomatic stimulation. These actions were suppressed by iontophoretic STY and PTX, respectively. In accordance with previous experiments (ibid.), IPSPs (peak latency 40 msec) induced by monopolar stimulation (4 pulses, 800 Hz, 30--60 microAmps) within the NGC were observed during AS. STY, when ejected prior to or during the onset of AS (30--200 nAmps, 30 sec--2 min) markedly reduced the amplitude of these AS potentials or blocked them completely (n = 10), whereas PTX (75--230 nAmps, 30 sec--2 min) had no effect (n = 10). To the extent that STY is considered a specific GLY antagonist, these data suggest that GLY may be involved in mediating NGC-induced IPSP activity in lumbar motoneurons during AS.

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216.8 NEURONS RELATED TO VOCALIZATION IN THE MONKEY PERIAQUEDUCTAL GRAY. C.R. Larson,* and M.K.Kistler*(SPON:L. Halpern). Dept. of Communicative Disorders, Northwestern University, Evanston, Illinois, 60201

The midbrain periaqueductal gray (PAG) has been shown to be important for vocalization. Electrical stimulation of the PAG in many animals elicits vocalization, while PAG ablation leads to mutism. In order to better understand how PAG mechanisms are involved in vocalization, extracellular spikes from 86 neurons in a small part of the dorsolateral PAG were recorded in monkeys trained to vocalize using operant techniques. Forty eight neurons appeared related to vocalization. The exact activity patterns were somewhat variable. Some cells were inactive and only began discharging prior to vocalization. Other cells were sporadically active but always increased activity before vocalization. The latency from unit onset to vocalization was quite variable, ranging from 1 sec to 70 msec. Cell discharge rates reached a peak near the onset of vocalization or shortly thereafter and rapidly became quiet after vocalization. Other units were observed that appeared to be related to other types of oral-facial behaviors, such as tongue activity.

In order to determine if the cells were projecting to the laryngeal system, spike triggered averaging and microstimulation techniques were employed. Activity from the laryngeal muscles was recorded from surgically implanted EMG electrodes. For most of the units tested, averaged EMG activity increased near the time of the triggering unit, suggesting a laryngeal projection. Microstimulation caused activation of laryngeal EMG in only a few cases, suggesting most PAG cells do not project to the laryngeal system. The discrepancy in results may be explained by the fact that many PAG cells become active in near synchrony, and hence are temporally correlated with vocalization even though they may project to other muscle systems.

The PAG recieves projections from other vocalization areas of the brain such as the anterior cingulate gyrus and amygdala and projects to several cranial nerve nuclei including nucleus ambiguus. The PAG may function as a relay between higher and lower areas of the brain involved in vocalization, as a premotor coordinating center, or it may be involved in initiation of vocalization. Other investigators (Jürgens and Pratt, 1979) have suggested the PAG may couple various motivational states to a particular type of vocalization.

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216.9 ORGANIZATION OF THE FACIAL MOTOR NUCLEUS IN MACACA FASCICULARIS. C. Welt, J.H. Abbs*, and R. DePaul*. Speech Motor Control Labs., Waisman Center, Univ. of Wisconsin, Madison, WI 53705-2280.

The neural mechanisms underlying motor control of the facial muscles have received little attention, despite the prominent role of facial gestures in both human and nonhuman primate communication. In this study we determined the musculotopic and morphologic organization of the facial nucleus (FN) in M. fascicularis. In a separate abstract we describe morphologic and histochemical characteristics of some facial muscles from the same animals (Sufit, R. et al., 1984, Neurosci. Abstr., 10).

Individual muscles were surgically exposed under an operating microscope and injected with horseradish peroxidase (HRP) conjugated with cholera toxin. Injections of 1.0 µl were made at multiple sites with a Hamilton microsyringe and 30 g needle. No reflux of HRP was seen. Transverse serial sections were processed with the tetramethyl benzidine protocol, and every other serial section was counterstained.

In M. fascicularis, the FN extends rostrocaudally for about 2.0 mm through the medulla and pons. The nucleus is round to oval in shape with a mean diameter of 1.0 mm. Cytoarchitectonically, a number of subnuclei could be distinguished but their boundaries were not sharp or consistent between successive sections.

Retrograde labeling of FN neurons was seen only on the ipsilateral side. The distribution of labeled motoneurons supplying individual muscles showed a musculotopic organization. Orbicularis oris inferior and orbicularis oris superior motoneurons were distributed differentially in the lateral region of the FN, while motoneurons innervating extrinsic ear muscles were located medially. Within this organization, labeled motoneurons for an individual facial muscle were distributed through almost the entire rostrocaudal extent of the FN. Dendritic processes of FN motoneurons were extensively labeled with the HRP cholera toxin conjugate. These dendritic arbors branched profusely and often projected into adjacent subnuclei. Occasionally, some branches extended outside of the FN as defined in Nissl sections. Quantitative analyses of neuron size in the FN revealed a mean diameter of 23 µ and a range of 12-35 µ. Inasmuch as most of the labeled motoneurons were relatively large, these data suggest that there may be more than one functionally distinct population of cells in the FN.

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216.10 CONTROL OF VIBRISSAL MOVEMENT BY THE FACIAL NERVE IN THE RAT. Kazue Semba and M. David Egger. Department of Anatomy, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854.

Rhythmical whisking of the vibrissae at about 7 Hz during exploration is one of the most conspicuous behavioral patterns in the rat. Although the sensory input from the vibrissae has been analyzed extensively, relatively little has been known about the motor control of the vibrissae. For example, vibrissal movement is actuated by the muscles around the muzzle, which have been thought to be innervated by the facial nerve. However, the exact branches of the facial nerve involved were not known. To investigate this question, the individual motor branches of the facial nerve including the buccal, marginal mandibular, cervical, posterior auricular, temporal, and zygomatic branches were cut, either singly or in various combinations. We found that vibrissal movement could be abolished only by transection of both buccal and marginal mandibular branches. In the rats in which only one of these two branches was cut, or in the rats in which the other branches were cut, vibrissal movement was indistinguishable from that seen in unoperated rats.

To trace back the central origins of the buccal and marginal mandibular, as well as the other branches of the facial nerve, horseradish peroxidase (HRP) was applied to the cut proximal ends of these individual branches. The HRP labelling in the facial motor nucleus revealed topographical representation of these branches, in which the buccal and marginal mandibular branches were represented laterally. This is consistent with earlier studies with HRP injections into facial muscles. The motoneuronal population devoted to vibrissal movement did not seem to be substantially larger than that for other facial movement. No labelling was seen in the mesencephalic trigeminal nucleus.

To compare the morphology of buccal and marginal mandibular motoneurons with other facial motoneurons, the intracellular HRP injection technique was used. One antidromically identified buccal motoneuron was 35 µm in longest dimension, and had relatively aspinous and unbranched multiple dendrites. These dendrites exhibited a mediolateral orientation, and some of them, more than 350 µm in length, extended beyond the limits of the facial nucleus into the reticular formation. Supported by General Research Support grant from Rutgers Medical School and NSF grant BNS 8341050.

216.11 MORPHOLOGY OF NORMAL CAT HYPOGLOSSAL MOTONEURONS AS DETERMINED BY INTRACELLULAR HRP INJECTIONS. Etsuo Shohara* and Jonathan O. Dostrovsky, Department of Physiology, University of Toronto, Toronto, Ontario, M5S 1A8, Canada.

Little information is available on the morphology of hypoglossal motoneurons in the adult cat. In this study intracellular recordings were obtained from neurons in the hypoglossal nucleus of chloralose anesthetized cats. Neurons were identified by antidromic activation from the hypoglossal nerve. Horseradish peroxidase was iontophoretically injected into the cells. Six to 12 hours following the injection the animals were perfused with a buffered glutaraldehyde-paraformaldehyde fixative and the brainstem subsequently sectioned. The sections were processed according to the DAB method and counterstained with cresyl violet. 7 neurons were successfully labeled. The somata of 6 of the neurons were multipolar in shape and one was fusiform. All the somata were within the anatomically defined borders of the hypoglossal nucleus although their dendrites projected beyond the margins of the nucleus dorsally into nucleus intercalatus and the dorsal nucleus of the vagus and laterally into the adjacent reticular formation. No dendrites crossed the midline or extended ventrally into the paramedian reticular nucleus. The dendritic tree was relatively simple and had few branches. Many dendrites extended 500u or more from the soma. The mean dendrite diameter at distances of 30, 50, 80,and 100 microns from the soma surface were 2.5, 1.5, 1.2 and 1.0 microns respectively. No spines were evident on the dendrites although beaded swellings were evident on some dendrites near their distal end. The axon left the nucleus in a ventrolateral direction and followed a relatively straight course to the edge of the brainstem. No axon collaterals were seen. These findings indicate that the hypoglossal motoneurons appear to differ from spinal cord α-motoneurons in the absence of axon collaterals, a simpler dendritic shape, and smaller diameter dendrites which taper more rapidly.

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216.12 CORTICOMOTONEURONAL SYNAPSES: LIGHT MICROSCOPIC LOCALIZATION UPON MOTONEURONS OF INTRINSIC HAND MUSCLES IN THE MONKEY. D.G. Lawrence, R. Porter and S.J. Redman*, Experimental Neurology Unit, John Curtin School of Medical Research, Australian National University, Canberra 2601, Australia.

Preterminal and terminal axonal arborizations of individual corticospinal neurons have been visualized in cats (Futami et al., Brain Res. 164:279,1979) and monkeys (Shinoda et al., Neurosci. Lett. 23:7,1981) using intra-axonal injection of horseradish peroxidase (HRP). The arborizations ramified in laminae V, VI and VII and, in the monkey, additional collaterals extended into lamina IX where cell bodies and proximal dendrites of motoneurons (MN) with axons in major forelimb nerves had been retrogradely labelled with HRP. It was concluded that a single corticospinal axon could ramify in up to four motor nuclei and that the corticomotoneuronal (CM) terminals made "apparent contacts with proximal dendrites or distal dendrites of the motoneurons". Intracellular injection of HRP permits virtually complete visualisation of a neuron and in the present study this enabled the location of intra-axonally stained CM synapses to be demonstrated upon intracellularly stained MN of intrinsic hand muscles in the monkey.

Corticospinal axons activated by low threshold stimulation of the "hand" area of the precentral gyrus were impaled in the lateral funiculus at C_7-C_8. Following injection of 1 to 9 such axons, MN activated antidromically from the median or ulnar nerves at the wrist were filled by intracellular injection of HRP. Connections between CM fibres and MN were reconstructed from parasagittal 100 μm sections reacted by the cobalt-enhanced DAB method.

Fifty-six corticospinal axons and 71 MN were injected in 14 monkeys (M. fascicularis). Stem axons in the lateral funiculus gave rise to main collaterals which provided an extensive arborization within the gray matter. En passant and single or clustered groups of terminal boutons arose from shorter, finer preterminal branches of these arbors. Seven light microscopically identified CM contacts (0.6x3 μm to 2.4 x 3.6 μm) were found upon the dendrites of stained MN in two of the animals. The results indicate that each main collateral of a CM axon establishes very few synaptic contacts, and possibly only one, with the dendrites of recipient MN. The targets for the numerous other synapses could not be established but they provide the substrate for a wide divergence of influence to MN as well as to interneurons which could have excitatory or inhibitory effects upon MN.

216.13 EXTENSIVE BUNDLING OF MOTONEURONAL DENDRITES REVEALED BY CHOLINE ACETYLTRANSFERASE IMMUNOCYTOCHEMISTRY. R.P. Barber* P.E. Phelps, and J.E. Vaughn. Beckman Research Institute of the City of Hope. Duarte, CA 91010.

Dendritic bundles formed by somatic and autonomic motoneurons have been studied in immunohistochemical preparations of rat spinal cord with monoclonal antibodies to choline acetyltransferase (ChAT). In contrast to existing light microscopic methods, this procedure revealed most, if not all, motoneuronal dendrites, thereby allowing for a comprehensive description of their morphological relationships.

ChAT-positive (ChAT+) dendrites in all somatic motor columns exhibited some degree of bundling. Substantial, longitudinal bundles were observed within the ventrolateral, ventromedial and central motor columns. Particularly dense longitudinal bundling occurred in the dorsolateral motor column at spinal level L6. Transverse bundles of motor dendrites were present within all motor columns and also occurred between several different motoneuronal groups, including the central, ventromedial and ventrolateral, as well as the dorsomedial and dorsolateral groups at L6. Some transverse dendritic bundles from medial motoneurons crossed in the ventral commissure and intermingled with dendrites of corresponding contralateral cells. Lateral and medial longitudinal bundles of ChAT+ dendrites from sympathetic preganglionic neurons coursed throughout the entire intermediolateral (ILp) and central autonomic (CA) columns, respectively. Transverse dendrites of ILp neurons formed striking, medially-projecting bundles at periodic intervals that intermingled with similarly spaced, laterally-projecting bundles of ChAT+ dendrites from CA neurons. Together, the longitudinal and transverse bundles from ILp and CA neurons formed a ChAT+ ladder-like arrangement in the intermediate spinal gray matter that delineated the dorsal from the ventral horn. Parasympathetic neurons of the intermediolateral sacral nucleus also gave rise to longitudinal and transverse bundles of ChAT+ dendrites, but they were not as prominent as those in the sympathetic cell columns.

The results of this study indicate that extensive bundles of somatic and autonomic motoneuronal dendrites occur widely in rat spinal cord. Such structures could provide a robust substrate for potential ephaptic dendritic interactions and/or for maximizing synaptic convergence of various afferent systems upon relatively large groups of motoneurons. Supported by NSF grant BNS-8219831.

216.14 RAT LEVATOR ANI MOTONEURONS AS COMPARED WITH THOSE OF SOLEUS, EXTENSOR DIGITORUM LONGUS AND BULBOCAVERNOSUS AFTER RETROGRADE LABELING WITH INTRAMUSCULAR WHEAT GERM AGGLUTININ. R.D. Rose, and W.F. Collins,III. Dept. Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

The spinal nucleus of the bulbocavernosus (SNB) is a midline nucleus (L5-L6) consisting of motoneurons (MNs) innervating levator ani (LA) and bulbocavernosus (BC) muscles. These MNs are sensitive to circulating androgens (Breedlove & Arnold Brain Res 225; J. Neurosci 3). LA and BC MNs were identified in male Sprague-Dawley rats (250-400 g) by retrograde labeling with wheat germ agglutinin (WGA). Similarly labeled MNs from two hindlimb muscles (soleus, SOL; extensor digitorum longus, EDL) were examined for comparison and control. Briefly, 10 μl of 0.1% WGA was injected through a single hole into the muscle (minced within the sheath); care was taken that no label escaped the muscle sheath. Survival was 3-4 days. Animals were perfused with warm saline followed by cold 4% paraformaldehyde. Sections were cut at 40 μm. WGA was visualized by either indirect fluorescent or PAP IHC techniques. With these techniques extensive dendritic morphology was observed in all groups (tertiary dendrites were often clearly defined). All labeled MN profiles with nuclei were photographed and measured (somal cross sectional area) from images projected on a computerized digitizing tablet (double blind regime). LA and BC MNs were found clustered within the SNB. Such clustering was not observed with SOL and EDL MNs which were found in lateral MN pools. LA and BC MNs exhibited extensive medially projecting dendritic arborization; labeled dendrites were routinely observed to cross the midline and lie in close juxtaposition to dendrites and somata of contralateral SNB MNs. In contrast, similar dendritic organization was not observed for SOL and EDL MNs. When one LA muscle and the contralateral BC muscle were injected in the same animal, labeled crossing dendrites from both MN pools were juxtaposed to labeled dendrites and somata on the contralateral side. Means and size distributions (bimodal) of SOL and EDL MNs were similar (ANOVA, X^2, $p>.05$). LA and BC MNs (unimodally distributed) were smaller than EDL and SOL MNs (ANOVA, $p<.01$). It appears that the peaks of the distributions of LA and BC MNs may correspond to the smaller of the two peaks seen in SOL and EDL distributions. BC MNs were larger than LA MNs (t-test, $p<.01$). LA MNs from intact and castrated (>30d) animals were similar morphologically and were of similar size (ANOVA, $p>.05$). Interestingly, these MNs exhibited different size distributions (X^2, $p<.01$). Supported by NIMH MH08323(RDR), NIH NS06407 & NS 20264(WFC) and NS14899 & NS16996 to L.M. Mendell.

216.15 PUTATIVE MECHANORECEPTORS INTRINSIC TO THE SPINAL CORD. D.M. Schroeder, Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN 47405

Specialized cells at the lateral edge of the spinal cord of snakes, the marginal cells, are in intimate contact with ligaments that are attached along the total length of the spinal cord. The structure and organization of these cells and ligaments suggest that they act as mechanoreceptors. The spinal cords of Crotalus were treated with standard neurohistological techniques and examined at the light and EM level. The ligaments, one on each side of the cord, are attached to the cranium rostrally and separated from the glial cells, forming the external boundary of the cord, by only a basement membrane. Collagen fibers fill the ligaments, interspersed with fibroblasts and elastic fibers. Within each intervertebral area, however, all the collagen fibers disappear from the ligaments. At exactly this point, a specialized area becomes evident in the spinal cord just deep to the ligament. Eight to twelve large neurons are alined along the ligaments' border with processes that extend into a narrow space between the cell bodies and the ligament. These processes, filled with mitochondria, have finger-like projections. Surrounding these neuronal processes are processes of a different kind. They are of variable size but most often one larger one is accompanied by a number of smaller ones. Very small tubules are found within the larger processes whereas filaments are within the large as well as small ones. In a ongitudinal section these latter processes appear tubular and are arranged parallel with the long axis of the spinal cord and appear to originate from cells adjacent to the group of neurons. On the medial side of the neurons are smaller neurons, several myelinated axons and a strip of neuropil. Surrounding this cellular and neuropil area is the white matter of the spinal cord. Grillner et. al. (Science 1983) suggested that the edge cells in the lamprey's spinal cord serve as an intraspinal mechanoreceptor. Some of the marginal cells of Crotalus have the appearance generally described for mechanoreceptor cells and the structural changes of the adjacent ligament within the intervertebral area appear ideal to respond especially to the lateral movement of the vertebral column.

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CEREBELLUM II

217.1 THE PARAFLOCCULAR CORTICONUCLEAR PROJECTION IN THE RAT CEREBELLUM. R.A. Burne, G.M. Mihailoff and D.J. Woodward. Bendix Advanced Technology Center, Columbia, MD, 20145 and UTHSC, Dallas, TX, 75235.

Previous work from our laboratory has indicated the paraflocculer lobule (Pfl) of the rat is a cerebellar target lobule for visual information. This study was undertaken to identify those regions of the deep cerebellar nuclei which receive projections from the Pfl. A specific issue addressed was whether the Pfl projects directly to vestibular nuclei. The techniques of orthograde transport of tritiated amino acids and horseradish peroxidase (HRP) were employed to delineate the output projection from the Pfl.

Injections of tritiated leucine and proline into to the dorsal, ventral and accessory subdivisions of the Pfl resulted in labeling preterminal and terminal axonal processes over the small and large cell divisions of the lateral and interpositus cerebellar nuclei (Lt and Ip, respectively). Autoradiographic grain distributions were restricted to ventrolateral regions of Ip and Lt. Although there was considerable overlap between projection fields from individual sublobules, a topographic organization was evident. The accessory Pfl projected upon the most ventral zones of Lt, the ventral Pfl upon ventrolateral Ip neurons and more dorsal regions of Lt, and the dorsal Pfl to further dorsal regions of middle and lateral Lt and Ip. A rostrocaudal topography also was present where the rostral Pfl projected upon more rostral regions of the cerebellar nuclei. Injections of HRP into the Pfls resulted in staining axonal elements over similar regions of Lt and Ip.

No grain distributions were observed over vestibular nuclei except for the control experiments with injections involving the floccular lobule. HRP-labeled cells also were observed in Lt and Ip but not in vestibular nuclei. These findings indicate that the Lt and Ip are the target nuclei for paraflocculer efferents. Whether visual information may project upon these deep cerebellar nuclei via paraflocculer Purkinje cells or collaterals of pontoparaflocculer fibers is an issue for future investigation.

217.2 TOPOGRAPHICAL AND FUNCTIONAL PROPERTIES OF LATERAL CEREBELLAR OUTPUT IN THE RAT. AN ANATOMICAL AND ELECTROPHYSIOLOGICAL STUDY. S. Ausim Azizi, R A Burne, J K Chapin and D J Woodward. Dept. Cell Biol, Univ Texas Health Sci Ctr at Dallas, Texas 75235

We recently demonstrated that the visual and auditory cortices project to paraflocculus via the cortico-pontocerebellar pathways. In this report we present data describing projections of the paraflocculus and the lateral cerebellar cortex onto the deep cerebellar nuclei and in turn projection of these nuclei to the thalamus and the cerebral cortex. The techniques of 1) anterograde transport of labeled amino acids, 2) single unit recordings from the deep cerebellar nuclei, and 3) stimulation of these nuclei and recording from sensorimotor cortex in anesthetized and awake behaving animals were employed.

Hydraulic injections of (.2-.3 µl) of 3H-leucine (77 mCi/ml) into the paraflocculus and the crus I and crus II resulted in afferent terminal labeling over specific regions of the ipsilateral lateral and interpositus nuclei of the cerebellum. Autoradiographic silver grains were primarily localized over the ventral caudal regions of the large cell subdivision in the lateral and interpositus nucleus and over the small and large cell subdivision in the ventral caudal interpositus nucleus.

Single unit recordings were obtained from identified neurons in the lateral and interpositus nuclei of halothane anesthetized rats. Histogram analysis showed evidence of excitatory and/or inhibitory inputs following electrical stimulation of the visual and auditory cortices with latencies ranging from 3 - 15 ms. Electrical stimulation of lateral and interpositus nuclei evoked multiunit and single unit responses in the sensory and motor cerebral cortices. In awake behaving animals, differential excitatory and inhibitory responses were observed in the activity of motor cortical neurons during rest or treadmill locomotion.

These obsrvations indicate that sensory inputs to the paraflocculus along with those to the crus I and crus II of the lateral cerebellum modulate other motor and sensory areas of the CNS. Analysis of the output properties of the paraflocculus may serve as a useful model for understanding of the role of many subregions of the lateral cerebellum in the sensorimotor integration. (Supported by NIAAA grant 3901, DA 02338 and the Biological Humanics Foundations.)

217.3 ORGANIZATIONAL TOPOGRAPHY AND CHARACTERISTICS OF THE NUCLEOCORTICAL PROJECTION OF THE CAT CEREBELLUM. A. Legendre* and J. Courville. (SPON: R. Dubuc). Centre de Recherche en Sciences Neurologiques, Département de physiologie, Université de Montréal, C.P. 6128, Montréal H3C 3T8

The nucleocortical projection terminates as mossy fibers in the granular layer of the cerebellum. It has been studied with the method of anterograde transport of L-leucine of high specific activity (130-147 Ci/mmol) in the cat. Survival times varying between 9 and 15 hours which are appropriate to demonstrate the terminal glomeruli were used. Small injections were placed in each of the cerebellar nuclei and the efferent cerebellar projections to the brainstem served as controls of the autoradiographs in all cases.

In the nucleus interpositus anterior (NIA) injections located within the rostral half of the nucleus were obtained. In these cases, there were no fibers of passage in the white matter nor terminals in the cortex and it is probable that the NIA does not provide any nucleocortical fibers. For the other nuclei, the projections were always sparse and appeared as localized clusters of grain deposits separated by empty zones. The overall areas of cortex impinged upon by the nucleocortical projections never involved more than a fraction (at the most 30%) of the cortical surface. After an injection in the caudal half of the nucleus medialis (NM) bilateral distributions of grain deposits were located in lobules VI and VII. Some deposits were also seen in lobules I, V and VIII. In the other two nuclei, the projections were unilateral. Injections involving the nucleus interpositus posterior (NIP) demonstrated terminals in lobule VI, crus I, the paramedian lobule, the paraflocculus and the flocculus. Scattered deposits were also found in crus II and in the paramedian regions of lobules II, III, IV and V. Following injections restricted to the nucleus lateralis (NL), crus II and the flocculus presented the largest number of terminals. Less dense distributions were found in crus I and lobules V and VI. There are distributions within the same lobules of projections from the NM and NIP or from the NIP and NL. However, it is not possible at present to decide whether there is an actual overlap of the terminals from two of the nuclei or separate complementary distributions.

Supported by a grant from the Canadian Medical Research Council.

217.4 LOCALIZATION OF SEROTONIN IMMUNOREACTIVITY IN THE DEEP CEREBELLAR NUCLEI OF THE OPOSSUM. G.A. Bishop, R.H. Ho and J.S. King. Department of Anatomy and Neuroscience Research Laboratory, The Ohio State University, Columbus, Ohio 43210.

We have used the PAP technique to describe: 1) the course of serotonin (5HT) fibers to the cerebellar nuclei 2) the differential distribution of 5HT within the nuclei; and 3) the continued course of these fibers to the cerebellar cortex. Few if any 5HT fibers are found in either the restiform body or the brachium pontis. However, a distinct bundle of 5HT fibers is present in the medial aspect of the brachium conjunctiva (BC). These fibers enter the cerebellum ventromedial to the nucleus interpositus anterior (NIA) and course posteromedially. Upon reaching the caudoventral aspect of the nuclei, the main fiber bundle turns abruptly dorsalward. Fibers arise from this ventral bundle and course dorsally into the neuropil of the deep nuclei. The densest immunostaining is seen in posterior and ventral regions of all four cerebellar nuclei. Anteriorly only sparse to moderate 5HT immunolabeling is seen. Within the nuclei, small diameter (0.25-0.5 μm) varicose fibers tend to orient primarily in a mediolateral direction. In NIA and the ventrolateral aspect of the fastigial nucleus (FN), discrete 5HT labeled laminae interdigitate with 5HT free bands. The innervation of the cerebellar cortex is derived from fibers that course through the deep nuclei in a specific manner. Fibers radiating from the lateral aspect of the dentate nucleus are directed toward lateral portions of the cortex. Fibers continue from the rostrodorsal pole of the NIA to anterior vermal lobules. Posterior to the primary fissure a discrete plexus arises from the dorsal aspect of the FN and courses toward the base of lobules V & VI. At levels caudal to the deep nuclei a single midsagittal band courses into lobules VIII & IX. Vermal lobules I and X receive 5HT fibers directly from the ventral fiber bundle. In conclusion, 5HT afferents enter the opossum cerebellum in the brachium conjunctiva and distribute differentially within the deep nuclei. The densest labeling is seen in regions that primarily contain small neurons. Physiologically, many of these neurons have been shown to have axons that collateralize and project to the thalamus, inferior olive and cerebellar cortex (Tolbert et al., '78; Exp. Brain Res., 31:305). Finally, there is regional organization in the course of 5HT fibers through the deep nuclei to specific regions of the cerebellar cortex. (Supported by NS-18028, NS-17080, NS-08798. We thank Dr. R. Elde for the 5HT antibody).

217.5 DEVELOPMENT OF SEROTONIN (5HT) IN THE OPOSSUM CEREBELLUM. J.S. King, R.H. Ho and G.A. Bishop. Department of Anatomy and Neuroscience Research Laboratory, Ohio State University, Columbus, Ohio 43210.

Immunohistochemical analysis (King et al., '83 Neurosci. Abst., 9:1092) has revealed that 5HT fibers differ in the density of their distribution to the cerebellar lobules and layers in the adult opossum. In the present study we have used the indirect antibody peroxidase antiperoxidase technique to analyze the ontogeny of 5HT in the cerebellum of pouch young opossums ranging in age from postnatal day (PD) 1 to PD 50. During the course of development, 5HT fibers appear to enter the cerebellum via two spatially and temporally separate pathways. At birth (PD 1) a distinct bundle of serotonin fibers courses through the tectum to a point where the tectum fuses with the cerebellar anlage. There they enter the cerebellum and distribute to the medial dorsal aspect of the intermediate zone. At PD 11 fewer 5HT fibers course to the cerebellum via the tectal route and by PD 16 they are no longer evident. Serotonin elements are now present in a brainstem cerebellar continuity around the lateral recess of the fourth ventricle. These fibers distribute to ventral lateral aspects of the cerebellar plate. Between PD 1 and PD 7, 5HT elements in the cerebellum are first located in the intermediate zone which has been reported in other species to contain migrating cells from the ventricular zone. These cells differentiate to form Purkinje cells, deep nuclear neurons and Golgi neurons. Throughout later developmental ages, PD 11 - PD 50, 5HT fibers are present in the cellular zone of migration between the Purkinje cell layer and deep nuclei, the Purkinje cell layer, the internal granule cell layer and in the deep cerebellar nuclei. The external granule cell and the molecular layers rarely contain 5HT fibers. The presence of 5HT elements in the intermediate zone early in cerebellar development supports the suggestion that serotonin plays a role in neuronal differentiation (Lauder & Krebs '78, Dev. Neuroscience: 1:15). In addition, the early arrival and course of 5HT axons suggest that they do not follow pre-existing cerebellar pathways. For example, spinal afferents enter the cerebellum by PD 7 at the tectocerebellar junction (Martin et al., '83, Anat. & Embryol.: 166:191) and efferents from the deep cerebellar nuclei exit by PD 16 and form the brachium conjunctivum (Hazlett & Bagley, '83, Neurosci. Abst.: 9:871). (Supported by NS 08798, NS 17080, NS 18028. We thank Dr. R. Elde for the 5HT antibody).

217.6 ANATOMICAL EVIDENCE FOR THE PRESENCE OF GAD-IMMUNOREACTIVE AXONS, SYNAPTIC TERMINALS AND SOMATA IN THE BASILAR PONTINE NUCLEI OF THE RAT. G.A. Mihailoff and B.G. Border*. Departments of Cell Biology and Neurology, Univ. Texas Health Science Center, Dallas, Texas 75235.

The immunocytochemical visualization of glutamic acid decarboxylase (GAD), a key enzyme in the synthesis of the neurotransmitter, gamma-aminobutyric acid (GABA) is generally regarded as an appropriate means of demonstrating fibers, synaptic terminals and cell bodies of GABA-ergic neuronal elements in the CNS. Accordingly, we have applied this methodology to the rat brainstem and here report the presence of GAD-immunoreactive axons, synaptic terminals and somata within the basilar pontine nuclei (BPN).

Adult Long-Evans rats were perfused with a variety of different fixatives in order to determine the optimal conditions for the immunocytochemical staining of GAD-containing neuronal elements in the BPN. Free-floating vibratome sections were incubated with sheep GAD antiserum S3 which was kindly provided by Dr. D.E. Schmechel. In all cases, the immunoperoxidase (PAP) method of Sternberger was used to visualize GAD-containing elements in the neuropil. Our principal findings in these studies are the following. (1) A substantial number of GAD-positive axons and axon terminal fields are present diffusely throughout the entire rostrocaudal extent of the BPN, but are densely concentrated in dorsolateral, ventrolateral and ventromedial pontine regions. Immunoreactive axons are present among fibers of the cerebral peduncle but also appear to descend into the BPN from regions dorsal to the medial lemniscus. (2) *In contrast*, the number of unequivocally stained BPN somata is quite minimal. GAD-positive somata are scattered throughout the BPN but are seen in greatest frequency within those dorsolateral and ventrolateral pontine regions which also contain a high density of GAD-stained axon terminals. A number of stained somata also appear adjacent to the midline at rostral levels and within the reticulotegmental nucleus just dorsal to the BPN. (3) Preliminary EM studies clearly demonstrate GAD-positive axons and synaptic terminals in the BPN, and further studies will seek to clarify the source of such axon terminals. In this regard, the present observations suggest that some may arise extrinsic to the BPN while the presence of GAD-stained somata in the BPN could represent a system of GABA-ergic neurons whose axon distributes at least in part within the BPN. Supported by NS 12644 and NSF80-04853.

217.7 ULTRASTRUCTURAL IDENTIFICATION OF DORSAL COLUMN NUCLEAR TERMINALS IN THE BASILAR PONTINE GRAY OF RATS. R.J. Kosinski and G.A. Mihailoff. Department of Cell Biology, Univ. of Texas Health Science Center, Dallas, TX 75235.

In order to identify the axonal boutons of dorsal column nuclear (DCN) projections to the basilar pontine gray (BPG), the DCN were ablated in 15 adult Long-Evans rats by either suction or electrocauterization. Following 1-14 day survival periods, the animals were sacrificed and the BPG processed through routine EM procedures.

Two groups of axonal boutons were identified as undergoing an electron dense type of degeneration. Although both formed asymmetric synaptic junctions, one type contained only round clear vesicles whereas the other exhibited a mixture of dense core and clear pleomorphic synaptic vesicles, as well as large, dilated cisternal or vesicular profiles and dense shrunken mitochondria. The former category of degenerating terminals was further divided into two subgroups on the basis that one underwent a rapid degeneration process becoming extremely dense by 5-6 days while the other subgroup exhibited a much slower process of degeneration which was further characterized by the occurence of a brief period of neurofibrillar accumulation early (1-3 days) in the degenerative process. Both subgroups of terminals ranged from 0.5-2.5 µm in size and formed multiple synaptic junctions with proximal and intermediate dendritic shafts and spines. In addition, several dendritic protrusions invaginated some of these boutons and received asymmetric synapses as similarly described for DCN axonal terminations in the thalamus (VPL). This similarity suggests that these BPG afferents may represent collateral branches of medial lemniscal axons.

The second major category of degenerative DCN terminals, which contained a mixture of clear and dense core vesicles, varied in size from 1.0-2.0 µm and only formed single asymmetric synaptic contacts. While these terminals contacted proximal dendritic shafts and somata, the majority synapsed with intermediate to small dendritic shafts. None, however, were found in contact with dendritic spines nor did they ever become dark and shrunken during the survival times employed. The identification of a variety of degenerating axonal boutons suggests that the DCN may be relaying various types of information to the BPG via axonal projections which arise from more than one type of neuron, as previously demonstrated for the cat (May & Berkley, Neurosci. Abst., '83).

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217.8 A COMPARISON OF SPINO-OLIVARY NEURONS THAT PROJECT TO THE MEDIAL AND DORSAL ACCESSORY OLIVES OF THE CAT. H.H. Molinari. Department of Anatomy, Albany Medical College, Albany, N.Y. 12208.

Three distinct targets of the spino-olivary projections are the caudal portion of the medial accessory olive (MAO), the caudal dorsal accessory olive (DAO), and the rostral DAO. It has been suggested that separate populations of spinal neurons project to each of these target areas (Armstrong and Schild, The Inferior Olivary Nucleus, 1980). This possibility was investigated in the present study, which uses some data from a recent publication (Molinari, J.Comp.Neurol., 1984, 223, 110). Injections of WGA-HRP were made in three loci of the cat inferior olive (IO), as defined by examination of the injection sites and the climbing fiber labeling in the cerebellar anterior lobe: 1) caudal MAO which projects to zone a; 2) caudal and rostral DAO which project to zones b and c_1/c_3; and 3) caudal MAO and caudal DAO which project to zones a and b. A 48 hr survival time and TMB were used to visualize retrogradely labeled neurons in cord segments L5-S1. Many of the injections included portions of the adjacent reticular formation; control injections that included these regions but not IO were evaluated.

The vast majority of labeled neurons were found in the contralateral cord in three distinct populations: 1) in the dorsal horn, particularly the central portion of lamina V; 2) in the ventromedial ventral horn, along the medial border of laminae VII and VIII; and 3) scattered in the lateral funiculus. Injections in caudal MAO resulted in labeling of ventromedial neurons throughout segments L5-S1. Injections in DAO, either caudal or rostral and caudal, resulted in labeling of neurons in the ventromedial region of segments L7-S1, the lateral funiculus of segments L6-L7, and the dorsal horn of segments L5-L6. As the amount of rostral DAO in the injection increased, the dorsal horn labeling extended caudally into segments L7 and S1. No other changes were seen.

The three populations of spino-olivary neurons do not project to separate IO targets. Nevertheless, the IO target regions do not receive identical input from the spinal cord. Rather, the input to any one region reflects a particular segmental sampling of one or more populations of spino-olivary neurons. (Supported by NIH Grant NS-17693).

217.9 LIGHT MICROSCOPIC STUDY OF THE INFERIOR OLIVARY COMPLEX IN THE ADULT STAGGERER MUTANT MOUSE. G. J. Blatt* and L.M. Eisenman. Daniel Baugh Institute of Anatomy, Jefferson Medical College, Philadelphia, PA 19107.

In the homozygous mutant mouse, staggerer (sg/sg), approximately 60-90% of its cerebellar Purkinje cells (PC) are absent (Herrup and Mullen, 1979). Since cells of the inferior olivary complex (IO) project to the cerebellar cortex as climbing fibers and PCs provide their main postsynaptic target, it would be extremely interesting to determine the status of the IO in the adult staggerer. We can see if the loss of the primary target has altered either qualitatively or quantitatively aspects of the sg/sg IO. We report here on the results of a light microscopic examination of the IO. The morphology of the three major olivary subdivisions, the principal olive (PO), dorsal accessory olive (DAO), and medial accessory olive (MAO) were analyzed and IO cell counts were performed in three homozygous staggerer mice (B6C3) according to the method of Konigsmark (1970).

The organization of the IO in staggerer is somewhat preserved with all three major subdivisions identifiable in some sections. The PO is reduced but the ventral and dorsal lamellae are visible in some mid-rostral sections. The border between the PO and DAO is obscure in some sections and the rostrocaudal extent of the PO and DAO is decreased. The DAO is absent in many caudal to intermediate sections and greatly reduced in others. The MAO appears somewhat normal but in caudal sections is compact medially and reduced laterally. Subnucleus "b" appears absent or reduced and no distinct grouping of cells representing the dorsomedial cell column is observed. The rostrocaudal dimension of the staggerer IO is reduced by 25% but the mediolateral extent is similar to that of the normal mouse.

The most striking abnormality of the IO in the homozygous staggerer is that only approximately 40% of the IO cells are present (mean = 15,500) when compared to counts in the normal mouse (mean = 37,000). This result is not surprising since about 75% of the cerebellar PCs are also missing in this animal. No significant differences in IO counts are found in the left and right IO in sg/sg, even though some sections appear structurally asymmetric.

These observations show that the staggerer gene either directly or indirectly affects the organization and number of IO cells in sg/sg. It is unknown whether IO cells are, in addition to PCs, a primary site of gene action or secondarily affected due to the cerebellar malformation.

217.10 Direction and Position Coding of Saccade Related Neurons in the Cerebellar Vermis of the Cat
Paul M. Gochin* and James G. McElligott, Dept. of Pharmacology, Temple University School of Medicine, Philadelphia, PA., 19140

Previous studies have investigated Purkinje cell activity in the vermal cerebellum of alert animals (cats and monkeys) trained to make saccades along the primary axes. In the present study, we wished to examine more extensively two important properties of saccades (direction and position) with which Purkinje cell activity has been correlated. Head restrained cats made saccades to spots of light presented on a tangent screen in order to obtain a liquid reward. Two experimental protocols were used. The first paradigm served to evoke a 360 degree set of radial saccades originating from the same central position. In the second paradigm, animals were required to produce a matrix of eye movements made up of 40 horizontal and 40 vertical saccades covering an area of 20 x 20 degrees. Results from the first experiment (direction-radial paradigm) demonstrated that 80% (32/40) of related cells had a directional preference that was relatively broad (89% > 90 deg and 50% > 180 deg). In addition, a few cells (20%) responded to saccades made in all directions of movement. Results from the second experiment (position-matrix paradigm) showed that most of the cells (69%; 11/16) correlated with saccades that were made in discrete regions of the eye movement field. These cells possessed a directional preference that was maintained throughout the response region. These regions were relatively large usually occupping 30 to 50 percent of the oculomotor range that we investigated. The experimental results demonstrate that the response of individual Purkinje cells is correlated with saccades made in specific directions within prescribed regions of the oculomotor field. Each cell is broadly tuned (with respect to saccade direction) and the area where it responds is relatively large. It is possible that this information is used by the brain stem oculomotor complex to make position dependent corrections in order to compensate for non-linearities within the oculomotor system. This is in agreement with prior studies which show that cerebellar lesioned animals produce saccades with targeting errors that are position dependent.

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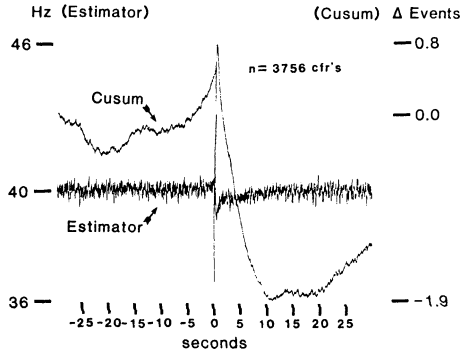
217.11 SECONDS-LONG CHANGES IN PURKINJE SIMPLE SPIKE ACTIVITY ACCOMPANY SPONTANEOUS CLIMBING FIBER DISCHARGES. C.C. Boylls, Jr., J.H. Onyski*, and L.A. Cohen*. Rehabilitation R&D Center, VA Medical Center, Palo Alto, CA 94304.

Purkinje cell simple spike activity is known to be reduced or suppressed for many seconds following repetitive electrical excitation of olivocerebellar pathways. Likewise, olivary microstimulation elicits enduring postural "biasing" of locomotor and oculomotor performances. Do *naturally occurring* climbing fiber responses (cfr's) also occasion lengthy simple-spike alterations? We have examined this issue through optimal linear estimation of the simple-spike activity surrounding spontaneous cfr's. The estimators were derived from time-extensive cfr/simple-spike cross-correlations (lags to ± 120 sec; resolution to 0.1 msec) in Purkinje cells recorded continously for up to 3 hours. Cells were studied in Voogd's zones B and C1, lobule V, of pre- or intercollicularly decerebrate cats, some generating stepping.

Illustrated below is such an estimator and its cumulative sum (the running integration of the estimator less its pre-cfr mean). The classic Purkinje "inactivation" pause and subsequent rebound are compressed into tiny spikes near 0 sec. Thereafter, simple spike activity is depressed by several Hz and requires roughly 10 sec to recover. Each of 26 units exhibited a similar seconds-long statistical alteration (usually a reduction) of activity associated with the cfr. Because alterations often began prior to the cfr (as in the cusum below), olivocerebellar activity alone may not *determine* such responses. However, it seems increasingly evident that climbing fiber volleys do indeed accompany protracted alterations in Purkinje simple-spike activity. **(Supported by VA Medical Merit Review.)**



217.12 REDUCTION OF CEREBELLAR NOREPINEPHRINE ALTERS CLIMBING FIBER ENHANCEMENT OF MOSSY-PARALLEL FIBER INPUT TO THE PURKINJE CELL. J.G. McElligott, T.J. Ebner, J.R. Bloedel, Dept. of Pharamcology, Temple U., Philadelphia, PA 19140, Depts. of Neurosurgery and Physiology, U. of Minn., Mpls., MN 55455

The Purkinje cell receives inputs from three discrete sources, the mossy fiber, the climbing fiber, and the noradrenergic (NE) fiber system. Each system is thought to serve a separate function in the processing of information by the cerebellum. Most studies have focused on interactions between the mossy and climbing fiber systems independent of the NE system. Previous work has shown that the responses of Purkinje cells to naturally evoked mossy fiber inputs are enhanced by the action of the climbing fiber system. These experiments investigated the effects of reduced cerebellar NE on this interaction. Recordings were made from 58 individual Purkinje cells in 3 chronic cats that were paralyzed and artificially respirated during the recording sessions. Previously, these animals received an intracisternal injection of 6-OHDA (ave. dose size - 1.65 mg) which effectively reduced the norepinephrine content (measured by HPLC-EC) of the cerebellum to 18% of control values. Purkinje cell simple and complex spike histograms to an ipsilateral forepaw tap were constructed. Unlike the previous studies in undepleted animals, inhibitory simple spike responses (42 components) evoked by a forepaw tap were enhanced when complex spikes were concomitantly evoked. Most of the excitatory response components (n=47) were not enhanced by concomitant climbing fiber discharge. In a large number of Purkinje cells, the amplitude of excitatory response components was actually reduced. These data show that there is a selective effect on the enhancement of excitatory Purkinje cell responses in animals with reduced NE. Since most of the NE input to the cerebellum terminates on the Purkinje cell, and since NE modulates the actions of excitatory and inhibitory neurotransmitters directly on these neurons, it is possible that this selective effect is a result of a reduction of the noradrenergic input to the Purkinje cell. At the present time, an external cerebellar mechanism cannot be entirely excluded since intracisternal 6-OHDA decreases NE in other regions. This work was supported by NIH grant NS 09447 and a grant from the Ben Franklin Partnership.

217.13 DECONVOLUTION OF A SIMPLE SPIKE "SIGNAL" FROM THE BACKGROUND ACTIVITY. T.J. Ebner, Depts. of Neurosurgery and Physiology, U. of Minn., Mpls., MN 55455

A major problem in evaluating the activity of Purkinje cells is the "noisy" background simple spike discharge. In this study an analytical method to deconvolve the simple spike signal evoked by a stimulus from the background activity is described. In decerebrate, unanesthetized cats Purkinje cell activity was recorded extracellularly, constructing simple and complex spike histograms during a brief, passive ipsilateral forepaw flexion-extension movement. After determining the time period of the simple spike response a period of background discharge was defined, consisting of an equal time period usually just prior to the simple spike response window. The average, instantaneous firing frequency for each stimulus presentation for the background and response window was determined. The large trial to trial variability was revealed in plots of the response, background, or response to background ratio. The probability densities for the response and background based on the average instantaneous firing frequency for each stimulus presentation were obtained. Based on the assumption that the response is the sum of two independent random variables, the background and the signal evoked by the forepaw displacement, Monte-Carlo techniques were used to deconvolve the signal from the response. To test the validity of the deconvolution, the signal and noise probability densities were convolved to generate a "simulated" response density, which was statistically compared to the observed response density. Many of the deconvolved signal probability densities exhibited small variances when compared to the noise and response probability densities. The mean of signal distribution for many Purkinje cells was considerably smaller than the mean of the noise distribution. An initial evaluation of the signal distribution during stimulus presentations when climbing fiber input was evoked and when climbing fiber input was not evoked supported our previous finding that Purkinje cell responsiveness is increased by climbing fiber input. Being able to directly evaluate the signal removed from the background activity will permit a better understanding of how the cerebellar cortex processes afferent inputs. Supported by NIH grant NS 18338.

217.14 OCULOMOTOR RESPONSES TO MICROSTIMULATION OF THE POSTERIOR CEREBELLAR VERMIS IN THE MONKEY. T. Fujikado* and H. Noda. (SPON: R. DeVoe), Sch. of Opt., Indiana Univ., IN 47405.

Stimulation experiments on monkeys have suggested that saccadic eye movements can be elicited from a wide area of the posterior vermis (lobuli 5, 6 and 7). We discovered that when small stimulus currents (less than 30 μA) were used, the area was relatively small, including lobulus 7 and the posterior part of lobulus 6. In the present study, single units were recorded from monkeys in which a search coil had been implanted on one eye. Oculomotor responses from the posterior vermis were studied with the same microelectrodes stimulating systematically at 100 μM steps. The stimulus loci with respect to the cerebellar layers were identified physiologically from characteristic neural activity during experiments and later confirmed histologically. The responsive area corresponded fairly well to the structure from which saccade-related discharges were recorded. In the cortex, low threshold loci were found in the granular layers and the thresholds in the Purkinje cell layers were always higher than those for the other layers. Low threshold loci were also discovered in the white matter. Typical oculomotor responses were saccades with a horizontal component to the stimulation side. When the stimulus was applied near the midline, a vertical component predominated. The directions and amplitudes of eye movements varied depending on the stimulus loci as well as the stimulus intensities. Within the same locus, a higher intensity produced saccades of a larger vectorial amplitude, but the effects on the horizontal and vertical components were usually different. At most cortical loci, mainly the horizontal component increased, producing more horizontal eye movements; however, at other loci, both the horizontal and vertical components increased, producing larger oblique eye movements. The trajectories of the majority of eye movements were not straight. This was caused by differences in the latencies, durations and peak velocity times between the horizontal and vertical components of the responses.

It is likely that there are elements in the cerebellar cortex that drive the eyes in one direction either in the horizontal or vertical plane. These elements may be intermingled at a certain ratio within individual cortical loci. The directions and amplitudes of evoked eye movements may be determined by the numbers and combinations of the horizontal and vertical elements activated by stimulations. (Supported by NIH Grant EY 4063)

217.15 OCULOMOTOR RESPONSES TO MICROSTIMULATIONS OF THE FASTIGIAL NUCLEUS IN THE MONKEY. H. Noda, Y. Tamaki*, S. Murakami* and T. Fujikado*. Sch. of Opt., Indiana Univ., IN 47405

Saccadic eye movements can be elicited by stimulation of the posterior cerebellar vermis (lobuli 6 and 7) in monkeys. Low threshold loci were found in the granular layers and the thresholds in the Purkinje cell layers were higher. In the present study, the pathway for the responses to vermal stimulations was traced down to the level of deep cerebellar nuclei with the same microstimulation technique. Eye position was recorded with a search coil method. The target area for stimulation was identified during experiments from neural activity recorded with the same microelectrodes. The responsive loci (eliciting oculomotor responses with currents less than 50 μA) were identified histologically from a marking spot of iron deposit at the deepest responsive loci of the track. Among 27 tracks, 24 were positively identified and the remaining 3 were reconstructed from the identified tracks. The oculomotor responses to deep cerebellar stimulations were saccadic eye movements. The latencies, durations and peak velocities of the responses were comparable to those observed during the vermal stimulations. The responsive loci were found in a relatively small area of the deep structure, including the posterior part of the fastigial nucleus and the white matter surrounding it. The white matter extending from the nucleus to the posterior vermis was also responsive. When the stimulus was applied to the fastigial nucleus and its vicinity, saccades toward the opposite side of stimulation were also observed. In the total of 275 responsive loci, stimulation of 157 loci (57%) elicited ipsilateral saccades and that of 118 loci (43%) yielded contralateral saccades. These locations were, however, intermingled. On the other hand, stimulation of the white matter extending toward the posterior vermis always produced ipsilateral saccades.

It is known that Purkinje cell axons from the vermis project to the fastigial nucleus and exert inhibitory synaptic action upon their target cells. Together with the observation that stimulations of the lateral portion of the posterior vermis elicited only ipsilateral saccades, it is likely that the contralateral saccades are the results of the activation of fastigial neurons, either at the cell bodies or at their axons. Therefore, the pathway for the oculomotor effects of the posterior vermal stimulations may involve the Purkinje cells in the responsive cortical loci.

(Supported by NIH Grant EY 4063)

217.16 AFFERENT PROJECTIONS TO THE AVIAN VESTIBULOCEREBELLUM. S.L. Freedman* (SPON: S.H. Robison). Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405

This study examined the inputs to the cerebellar auricle of the chicken (Gallus domesticus). The methodology of the present study differs from the previous report on the afferents to the lateral cerebellar appendage of primary folia IX and X of the pigeon (Brauth and Karten, Exp. Brain Res. 28:73-84, 1977) in two ways. First, the uptake and spread of the tracer enzyme was restricted by incorporation of horseradish peroxidase (HRP) in a polyacrylamide gel and second, the brain sections were stained with tetramethyl benzidine (TMB) for increased sensitivity to the peroxidase label. Dark field microscopy provided confirmation that the HRP-Gel implants were restricted to the auricular portion of the cerebellum.

Neuronal cell bodies were labeled in the rostral part the contralateral inferior olivary nucleus. The HRP positive nerve cell bodies in this complex were clustered medially in the dorsal lamella. A previously undetected cellular component was consistently identified within and immediately adjacent to all borders of the fasciculari longitudinalis medialis (FLM) at the ponto-medullary junction. Labeled neurons were also observed bilaterally in the pontine reticular formation at the levels of the cochlear and vestibular nerves. In the mesencephalon, neurons were identified bilaterally in the nucleus ectomamillaris (EM), which is also termed the nucleus of the basal optic root. A few responsive neurons were situated in the nucleus interstitialis of Cajal (IS). This nucleus is known to receive afferents from vestibular and accessory optic nuclei, but its participation in the relay of retinal projections to the vestibulocerebellum has not been reported previously.

The present findings provide additional information pertinent to the neuronal populations which project to the vestibulocerebellum in Aves. The current findings also have implications on the integration of the visual and vestibular systems in the cerebellum.

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217.17 CEREBELLAR UNIT RESPONSES TO TELENCEPHALIC INPUT IN CATFISH Luke T. Lee* (SPON: Charles E. Spooner). Neurobiology Unit, Scripps Inst. Oceanog. and Dept. Neurosciences, U.C.S.D., La Jolla, CA 92093.

Recent electrophysiological and anatomical studies have shown that the teleost cerebellum receives ample indirect input from the telencephalon. This input arises from area dorsalis centralis (Dc) of the telencephalon and distributes to a large area of the cerebellum.

In the present study, the effect of telencephalic (Dc) stimulation upon cerebellar unit acitivity has been examined in curarized catfish (Ictalurus nebulosus). Purkinje cells with simple spikes fire spontaneously at the rate of 8 to 50/s and respond to a single shock with an initial latency of about 40 msec. The response can consist of pure inhibition with a duration of 0.3-1.5 s; or initial inhibition with a duration of 0.05-0.2 s, followed by post-inhibitory rebound; or initial excitation followed by inhibition which may or may not be followed by postinhibitory rebound. Units responding with initial excitation are mostly found along the lateral edges and the midline of the corpus cerebelli; units responding with initial inhibition are most often found in the intermediate part of the corpus cerebelli. Changing the stimulation sites within Dc does not change the response pattern of the units but may alter their threshold intensities, latencies, and amplitudes.

Eurydendroid cells, the efferent neurons of the teleost cerebellum, also respond to telencephalic stimuation. They are recognized by their relatively long spike duration (12-20 ms), their low rate of spontaneous discharge (0.3-2/s), and their location (0.2-0.3 mm below the Purkinje cell region). They show either excitation with an initial latency of about 60 ms or inhibition followed by excitation and a second period of inhibition. The first response pattern is likely to be the result of direct activation by incoming cerebellopetal fibers. The second response pattern is probably elicited as the result of Purkinje cell action. Some of the units in this group have response pattern opposite to that of the Purkinje cells. However, the relation between Purkinje cell response and eurydendroid cell response is not necessarily simple.

Trains of electrical pulses with repetitive rates higher than 3/s also increase the spontaneous discharge of some Purkinje cells by as much as 100%. The effect may last several hundreds of seconds.

(Supported by NIH and NSF grants to Theodore H. Bullock.)

217.18 PROPAGATION OF ACTIVITY IN A DIRECTIONAL NETWORK WITH FEEDBACK: PARALLELS WITH THE CEREBELLAR CORTEX. M. Nahvi* and K. Doroudi* (SPON:F. Motamedi). Sharif University, P.O.Box 3406 Tehran, Iran

Branching of granule cell axons in cerebellar cortex propagates mossy afferent information in the longitudinal direction producing spatial summation in Golgi cells. Therefore inhibition of granule cells is affected by their neighbors activity.

In a synthetic network resembling the above chain, excitation is transmitted in x direction by direct paths of length ℓ. Feedback inhibition may also be transmitted in that direction. Result is propagation of afferent information beyond ℓ. In Fig. 1 input M and output P are n-dimensional vector functions of distance, x, and time, t, An incremental linear model with n=1 (Eq.1-a,b,c) and n=2 was simulated in unidirectional and bidirectional modes. Simulation was then extended to piecewise linear model with threshold and saturation.

Decisive factors in propagation depend on feedback, its time course, and delay. In the model of Eq.1 these factors are specified by h(t) and ℓ. The range and stability of propagation critically depend on the amount of feedback. i.e. magnitude of h(t) and length ℓ. An increase in the effectiveness of feedback facilitates propagation. Speed is governed by delay in feedback loop. For small delays speed approaches that of direct transmission. Waveform is controlled by the time course of feedback. A narrow afferent pulse remains narrow for small time courses.

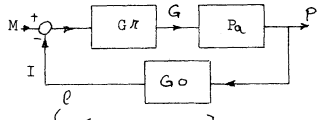


Fig.1

$$p(x,t) = \int_{-\ell}^{\ell} g\left[(x+\xi),(t-|\xi|)\right] d\xi \qquad \text{Eq. 1-a}$$

$$i(x,t) = \int p(x,\tau)\, h(t-\tau)\, d\tau \qquad \text{Eq. 1-b}$$

$$g(x,t) = m(x,t) - i(x,t) \qquad \text{Eq. 1-c}$$

218.1 RESPIRATORY MOTONEURON ACTIVITY IN THE ISOLATED LAMPREY BRAIN AFTER SPECIFIC LESIONS OF THE MEDULLA. K.J. Thompson. Dept. Physiology & Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

Fictive respiration is generated by bilaterally paired circuits of unidentified neurons in the lamprey medulla. Rhythmical discharges spontaneously occur in cranial nerves IX and X which supply gill musculature and cause exhalation (Rovainen, 1977, Fed. Proc. 36, 2386). These discharges result from the nearly synchronous activity of respiratory motoneurons (MNs) and occur in very brief bursts (50 msec duration). Respiratory activity can continue after complete midline section, but section between the trigeminal (V) region and the more caudal MNs eliminates respiratory discharges in cranial nerves. Respiratory activity can still be recorded extracellularly from coordinating interneurons at the midline of the isolated rostral medulla, demonstrating the presence of paired central pattern generators (CPGs) for respiration in the trigeminal region (Rovainen, C.M., 1983, Neurosci. Abst. 9, 541).

These CPGs produce brief strong EPSP bursts in respiratory MNs. The main excitatory input is eliminated after hemisection behind the ipsilateral trigeminal. There are also two other forms of rhythmical synaptic input to respiratory MNs: low amplitude, long duration (200-300 msec) EPSP bursts, and subsequent inhibition which consists of one or a few IPSPs. These inputs continue to occur in phase with respiratory bursts on the intact side after ipsilateral hemisection.

The IPSP input is selectively eliminated by contralateral hemisection behind the trigeminal region, or by section of the midline of the caudal medulla between the respiratory motor nuclei. This suggests that the periodic IPSPs originate contralaterally. Additionally, surface stimulation of the lateral trigeminal region causes IPSPs in contralateral respiratory MNs at the same threshold that produces EPSPs in ipsilateral MNs.

No specific lesion that was tested consistently eliminated the low amplitude, long duration EPSP bursts. Even after complete lesion of the trigeminal region of the medulla, rhythmical EPSPs could be recorded in respiratory MNs in some preparations. These findings suggest that the rhythmical low amplitude EPSP bursts are produced by a second set of CPGs, which are normally subordinate to those in the trigeminal region and which are located within the region of the respiratory MNs. Supported by NRSA NS 07154-02 (to K.T.) and USPHS NS 09367 (to C.M. Rovainen).

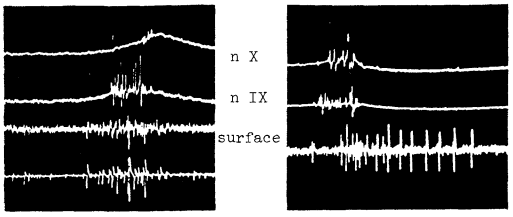
218.2 RESPIRATORY NEURONS NEAR THE TRIGEMINAL NUCLEUS IN LAMPREYS. David F. Russell. Comparative Neurobiology, CNRS - Univ. of Bordeaux, 33120 Arcachon, France.

Isolation experiments suggest that a pattern generator in the anterior medulla, duplicated on each side, drives the synchronous respiratory bursts in IX and X motoneurons via descending axons (Rovainen, Soc. Neurosci. Abstr. 9: 541, 1983).

Neurons bursting with the respiratory rhythm were classified from surface and intracellular recordings near nuc. V in isolated brain preparations from anadramous adult Lampetra fluviatilis. (I) Consistent with the above proposal, ventral surface records revealed spindle-shaped bursts starting 50-100 ms before the motorneuron discharges (Fig 1). Mild stimulation (3 μA, .5 ms) at this region evoked EPSPs in IX and X motorneurons bilaterally, as judged from motor root potentials, and strongly reset the rhythm with a phase response curve resembling that of endogenous bursters in other systems since a stimulus late in the interburst interval would trigger a burst and advance the timing of subsequent bursts. The axons of generator neurons may traverse this region. (II) Intracellular recordings were made from neurons that burst with the motorneurons and evoked time-locked EPSPs in them when stimulated with intracellular current. They may be pre-motor interneurons. (III) Surface records revealed a class of cells bursting after the respiratory motorneurons (Fig. 2, large unit). This is the first example of substantial asynchrony in the lamprey respiratory system.

In conclusion, several different types of respiratory neurons are located near nuc. V in adult lampreys, and may be part of the respiratory pacemaker.

Fig. 1 100 ms Fig. 2



218.3 THE STRUCTURE OF THE INTERSEGMENTAL COORDINATING SYSTEM OF THE LAMPREY CPG FOR LOCOMOTION. A. H. Cohen, Neurobiology and Behavior, Cornell University, Ithaca NY 14853.

The locomotor central pattern generator in the isolated lamprey spinal cord can easily be activated by D-glutamate to produce a stable frequency of bursting in all ventral roots (VRs). A typical feature of both intact swimming and glutamate induced bursting, is that there is a constant phase coupling between the activity in any two of the VRs. In the experiments reported here, 50 to 60 of the 100 total cord segments were used. When cut into 2 pieces having equal numbers of segments, the preferred frequencies of the two pieces almost always differed from that of the intact. In roughly two thirds of the cords, the rostral piece burst at a higher frequency than the caudal, in one third the caudal had the higher frequency, while rarely, the pieces had about equal frequencies. In the majority of the cords, the intact frequency was intermediate between the frequencies of the pieces after cutting. But the pieces could also both assume either higher or lower frequencies than the intact. Thus, the intersegmental coordinating system must be responsible for maintaining both a stable frequency of bursting in all segements as well as constant phase relations among segments.

Using the split-bath technique (Russell & Wallen, Acta. Physiol. Scand., 1980) the existence of long axon coordinating fibers is shown. While perfusing rostral and caudal regions with D-glutamate, the mid-ten segments of the 50-60 segment cord pieces could be perfused with saline containing $0Ca^{2+}$ high Mn^{2+}. This procedure did not destroy the coordination between rostral and caudal regions. By lesioning the medial or lateral fiber tracts in the midsegment in $0Ca^{2+}$, the distribution of long and short axon fibers was determined. Lesioning the lateral tracts and the gray cellular region of the midsegment in $0Ca^{2+}$ could uncouple the rostral and caudal segments. However, restoring normal saline restored coordination, demonstrating the presence of short axon coordinating fibers in the medial tracts. Lesions preserving the lateral tracts preserved the coordination across the ten segments in $0Ca^{2+}$. However, extending the lesion disrupted it; again, restoring normal saline restored coordination, pointing to short axon coordinating fibers in the lateral tracts. In summary, the long axon fibers are predominantly, but not exclusively in the lateral tracts. The short axon fibers are more distributed, being found in both medial and lateral tracts.

218.4 EFFECTS OF PROPRIOSPINAL INTERNEURONS ON FICTIVE SWIMMING IN LAMPREY. C.M. Rovainen, Dept. Physiology & Biophysics, Washington Univ. Medical School, St. Louis, MO 63110.

Three methods were used to test the roles of ascending and descending groups of propriospinal interneurons (PSINs) during fictive swimming induced by excitatory amino acids in the isolated spinal cord of adult lampreys.

(1) Extracellular recordings from the split ends of the spinal cord showed ascending and descending bursts in phase with contralateral or ipsilateral VRs.

(2) Electrical stimulation of the ipsilateral split caudal end of the hemisected spinal cord indirectly excited ascending PSINs (Symp. Soc. Exp. Biol. 37: 305) which enhanced and could entrain bursts in rostral contralateral ventral roots (VRs). Indirect electrical stimulation of descending PSINs diminished and could delay bursts in caudal contralateral VRs.

(3) With the method of Russell & Wallén (Acta Physiol. Scand. 108:9A) rostral and caudal compartments were bathed in D-glutamate or N-methyl(D,L)aspartate (NMA) with an intervening compartment with normal fluid or inhibited with glycine or GABA. (a) The caudal region was able to entrain bursts antiphasically in rostral VRs through separations of 20 segments in Ichthyomyzon or 35 segments in Petromyzon. (b) Inhibition of 1-2 segments by spot applications of glycine or GABA did not interrupt normal coordination or rostrocaudal phase lag. (c) Increased NMA in a rostral compartment could entrain VR bursts in an adjacent caudal compartment at a higher frequency and with stabilized rostrocaudal phase lag.

Two hypothetical types of PSINs are proposed for longitudinal coordination of fictive swimming: (i) Crossed, ascending INs which are excited in phase with nearby VRs and which excite and entrain rostral pattern generators on the opposite side, and (ii) short, crossed descending INs which are excited in phase with nearby VRs and which inhibit contralateral pattern generators.

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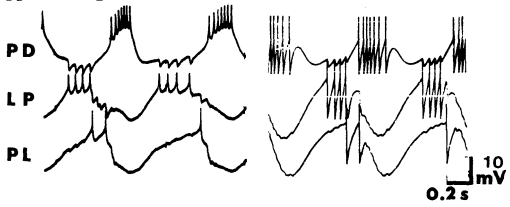
218.5 THIRD-GENERATION SIMULATIONS OF MOTOR PATTERN GENERATION IN THE LOBSTER STOMATOGASTRIC GANGLION. D.K. Hartline and D.V. Gassie*, Bekesy Laboratory, University of Hawaii, Honolulu, HI. 96822.

Physiologically based simulations have indicated that motor patterns in this ganglion cannot be explained by "conventional" cellular and synaptic properties, including even endogenous burst properties in PD/AB cells. We examined the effect of incorporating measurements of plateau properties (Russell & Hartline, J. Neurophysiol. 48: 914, 1982), chemotonic release (Graubard, et al. J. Neurophysiol. 50: 508, 1983), and A-conductance into simulations of stomatogastric cells.

Development of plateau currents appears significantly limited by cell RC properties. An activation characteristic of the form $g=\bar{g}(V-E)/(1+\exp((V_r-V)/w))$ was used for both inward (i) and outward (o) current mechanisms. Parameters were derived from measurements of rest potential (V_r), plateau threshold, plateau peak, input resistance and response to pulse perturbations. Repolarization parameters were derived from trough levels, resistances, and decay rates. For the example below (intrasomatic recording, left, from Graubard et al., 1983), values determined were $E_o=-90$; $E_i=40$; $V_r=-38$; $w=5mV$; $\bar{g}_o=2.4$ (rest units); $\bar{g}_i=2.4$, 2.2 and 2.1 in PD, LP, PL; repolarization $\bar{g}=1.8$, time-constant= 400 msec. The corresponding simulation (cellular parameters from Hartline, Biol. Cybern. 33: 223, 1979; PSP amplitudes measured directly) is shown at the right (referenced to trigger-zone). Substantial improvements in firing patterns, burst phasing and V_m trajectories were seen.

The simulations indicate that plateau properties may contribute significantly to timing as well as strength of bursts, and to the sensitivity of bursting cells to synaptic input. Chemotonic interactions may contribute to subthreshold regulation of trajectories and burst timing. A-conductances can contribute to phase delays and burst rate regulation. Discrepancies between model predictions and physiological observation point to further avenues for physiological investigation.

Supported by NIH NS15314.



218.6 MICROANATOMY OF SYNAPSES PROCESSING INPUT TO THE LATERAL GIANT AXONS OF THE CRAYFISH. Sun-Hee C. Lee and F. B. Krasne Department of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

The microanatomy of synapses in the circuit leading from primary afferents (PAs) to the dendrites of the lateral giant axons (LGs) of the crayfish central nervous system was examined and the findings compared to expectations from the well known physiology of the circuit.

From physiology it is thought that PAs excite LGs directly and via interneurons (INs) both of which make electrical synapses on the LGs. By contrast, synapses from PAs to INs appear to operate chemically. When LGs fire, PA terminals and LG dendrites near the main axon ("proximal" dendrites) are targets of GABAergic inhibition.

We found that the clear vesicles of cobalt labeled PA profiles were mostly less than 58 nM in diameter, whereas more that 30% of the vesicles in each IN profile sampled were larger than this. We therefore define two classes of presumably excitatory profiles: class I--those with less than 20% --and class II--those with greater than 20%--large clear vesicles. As expected, class I vesicles made primarily chemical synapses, defined by widened clefts, pre and postsynaptic densities, and presynaptic vesicle clusters, on INs. Both class I and II profiles synapsed on distal LG dendrites. The profiles synapsing on these dendrites made either chemical contacts or both chemical and electrical contacts. Contrary to expectation, pure electrical synpases on LG dendrites were almost never seen.

Both unspecified class I profiles and labeled PA terminals as well as both proximal and distal LG dendrites commonly received input from profiles containing narrow, often eccentric vesicles; these "class III" profiles are believed to be responsible for GABAergic inhibition. (Supported by NIH grant 08108)

218.7 ANALYSIS OF THE ROLE OF DESCENDING INPUT IN THE PRODUCTION OF HATCHING AND WALKING IN CHICKS: SPINAL TRANSECTION STUDIES. A. Bekoff. EPO Biology Dept., Univ. Colorado, Boulder, CO 80309.

This study is part of a series designed to examine the hypothesis that the same, or elements of the same, neural pattern generating circuitry is used to produce the leg movements of both hatching and walking. Previous studies in this series have shown that the circuitry for hatching is still present and functional in the posthatching chick and that bending the neck to the right or left serves as a specific signal to turn on the hatching leg motor output. Thus, the circuitry for the leg movements of hatching coexists in time with the circuitry for walking. This leaves open the possibility that it could be modulated in such a way as to produce the walking motor output.

Quantitative analyses have shown that there are basic similarities as well as some significant differences between the leg motor output patterns of hatching and walking. A recent study of posthatching chicks with deafferented legs (Bekoff, Neurosci. Abstr. 8:738, 1982) showed that some aspects of the hatching and walking leg motor output patterns became more similar after removal of sensory input. These results support the hypothesis that sensory input from the legs may normally modulate the output of one set of pattern generating circuitry in order to produce distinctive aspects of the motor output patterns that normally characterize hatching and walking.

Nevertheless, other aspects of the motor output patterns were maintained in the absence of sensory input from the legs. If the same circuitry is indeed used for both hatching and walking, then another source of modulatory input to be considered is input descending from higher levels. The present experiments have used 0- to 1-day old posthatching chicks with high cervical (C3) spinal transections in order to characterize the leg motor output patterns of hatching and walking in the absence of input descending from the brain.

Supported by NSF grant BNS 79-13826, NIH grant NS 20310 and a fellowship from the John Simon Guggenheim Foundation.

218.8 BLENDS OF SCRATCH REFLEX MOTOR PATTERNS ELICITED BY TRANSITION ZONE STIMULATION IN THE SPINAL TURTLE: SWITCHES AND HYBRIDS. L.I. Mortin, G.A. Robertson and P.S.G. Stein. Biology Dept., Washington Univ., St. Louis, MO 63130.

The red-eared turtle Pseudemys scripta elegans, when low-spinal, displays three different forms of the scratch reflex in response to tactile stimulation (Soc. Neurosci. Abstr. 8:159, 1982; Symp. Soc. Exp. Biol. 37:383, 1983). Each scratch form is distinguished from the other two forms by (1) its receptive field, (2) the portion of the hindlimb used to rub against the stimulated site and (3) the timing of knee extension in the cycle of hip protraction and retraction. In each scratch form, the limb rubs during activation of FT-KE, a knee extensor muscle. In the rostral scratch, the limb rubs with the dorsum of the foot during the latter part of activation of VP-HP, a hip protractor muscle. In the pocket scratch, the limb rubs with the side of the calf, knee and/or thigh during activation of HR-KF, a hip retractor muscle. In the caudal scratch, the limb rubs with the heel or side of the foot after the offset of HR-KF activity.

Transition zones (TZs) are narrow regions of the shell and skin that separate the boundaries of two scratch receptive fields. Based upon features (2) and (3) above, TZ stimulation elicits (i) a pure form of scratch, similar to a scratch form initiated within a receptive field next to the TZ, or (ii) a blend of scratch forms. Blends have characteristics of two different scratch forms. A blend is either a switch or a hybrid. A switch uses one rub form in one cycle and a different rub form in a successive cycle; a hybrid uses two different rub forms in each of several successive cycles. Rostral-pocket TZ stimulation produces blends utilizing the dorsum of the foot for one rub and the side of the knee and calf for the other. Two FT-KE activations can occur in these blends, one during the latter part of VP-HP activity and the second during HR-KF activity. Caudal-pocket TZ stimulation produces blends utilizing the heel or side of the foot for one rub and the side of the thigh for the other. Two FT-KE activations can occur in these blends, one after the offset of and the second during HR-KF activity. Blends are seen in recordings from both (i) the muscles FT-KE, VP-HP and HR-KF in moving turtles and (ii) the nerves innervating these muscles in turtles immobilized with neuromuscular blockade. Thus, the motor pattern generators in the spinal cord produce smooth blends in the absence of sensory feedback from the moving limb.

Supported by NIH Grants NS-15049 and NS-07057.

218.9 BLENDS OF SCRATCH REFLEX MOTOR PATTERNS ELICITED BY SIMULTANEOUS STIMULATION OF TWO SITES IN THE SPINAL TURTLE: SWITCHES AND HYBRIDS.
P.S.G. Stein, A.W. Camp*, G.A. Robertson, and L.I. Mortin.
Biology Dept., Washington Univ., St. Louis, MO 63130.

The red-eared turtle, when low-spinal, displays three forms of the scratch reflex in response to tactile stimulation (Soc. Neurosci. Abstr. 8:159, 1982; Symp. Soc. Exp. Biol. 37:383, 1983; Mortin et al., this volume). Stimulation of the mid-body shell bridge elicits a rostral scratch in which the dorsum of the foot rubs against the stimulated site. Stimulation near the tail elicits a caudal scratch in which the heel or the side of the foot rubs against the stimulated site. The rub occurs during knee extension for each scratch form. The site of the rub depends on the timing of knee extension in the cycle of hip protraction and retraction. During a rostral rub, FT-KE, a knee extensor muscle, is active during the latter portion of the activity of VP-HP, a hip protractor muscle. During a caudal rub, FT-KE is active after the offset of the activity of HR-KF, a hip retractor muscle.

Simultaneous maintained stimulation of two sites, one in the rostral and the other in the caudal scratch receptive field, elicits either (i) a rostral scratch, (ii) a caudal scratch, or (iii) a rostral-caudal blend. A blend is either a switch or a hybrid. In a switch, the limb rubs against one site in one cycle and then against the other site in the following cycle. In a hybrid, each of several successive cycles displays two rubs: one against the rostral site and the other against the caudal site. For all blends, the dorsum of the foot is used for each rostral rub and the heel or side of the foot is used for each caudal rub. During each rostral rub, FT-KE is active in the latter portion of VP-HP activity. During each caudal rub, FT-KE is active after the offset of HR-KF activity.

Stimulation of a single site in a turtle immobilized with neuromuscular blockade elicits an activation pattern in the nerves innervating FT-KE, VP-HP, and HR-KF that is similar to the activation pattern observed in these muscles in the turtle with limb movement. Simultaneous two-point stimulation in the immobilized turtle elicits motor patterns, including blends, similar to those observed in the moving turtle. The smooth blending of rostral and caudal patterns indicates that there is communication in the spinal cord between the rostral scratch generator and the caudal scratch generator.

Supported by NIH Grants NS-15049 and NS-07057.

218.10 JOINT MULTI-CORRELATION DISPLAY: A NEW PROCEDURE FOR ANALYSIS OF INTERACTIONS BETWEEN SIMULTANEOUSLY RECORDED NEURONS. R.D. Frostig and R.M. Harper, Neuroscience Program, Brain Research Institute, and Dept. of Anatomy, UCLA, Los Angeles, CA 90024.

Current neuronal recording procedures allow the acquisition of discharges from large numbers of simultaneously recorded neurons. There are, however, only limited procedures for examination of functional interactions between sets of neurons, especially if the number of cells exceeds 3 (Gerstein, G. L., et al., Brain Res., 140:43-62, 1978). Multiple cross-correlation, and joint impulse configuration scatter diagram ("snowflake"; Perkel, D. H., et al., Brain Res., 100:271-296, 1975) procedures are useful for examining simultaneous interactions between up to 3 pairs of neurons, but these analyses do not allow the examination of interactions between large numbers of cells. The following procedure, based on a technique by Inselberg (Inselberg, A., IBM LASC, G320-2711, 1981), allows examination of simultaneous interactions between a set of many neurons, with the number of cells analyzed within the set being limited only by practical constraints of the analytical machine.

The analysis consists of displaying density plots of cross-correlations between every combination of pairs of those cells comprising the set. Thus, a set of 4 cells ABCD will have cross correlations AB, BC, CD, AC, AD, and BD. These correlations are plotted vertically "on edge" with the amplitude of correlation coded as density or color. Each correlation is plotted in parallel time coordinates with the others. The occurrence of a strong time-locked relationship on one cross-correlation, i.e., AB, can be mapped to a corresponding time event in each of the other correlations, i.e., BC, CD, AC, AD, and BD, using lines of various densities (or colors) to indicate the degree of composite interaction (i.e., interactions that could not be predicted from cross-correlations). In practice, only the lines indicating the strongest composite interactions are displayed.

The procedure allows detection of discharge patterns between all neurons in many-celled sets. Thus, just as the display procedure for 3 cells allows examination of interactions that could not be predicted from paired comparisons alone, the joint multi-correlation display procedure allows us to obtain unique information on network relationships that could not be extracted from the analysis of fewer neuronal interactions.

Supported by HL22418-07 and AHA GLAA 678-1G3.

218.11 **FINE DISCRIMINATION AS AN EMERGENT PROPERTY OF PARALLEL NEURAL CIRCUITS.** **William H. Calvin,** Department of Neurological Surgery, University of Washington RJ-20, Seattle, Washington 98195.

A neuron is inherently noisy, in that the membrane potential baseline shows random noise from both internal and external sources. This affects the precision with which the membrane potential can represent an external variable; by causing jitter, it also affects the precision with which the neuron's pacemaker-like repetitive firing properties can code information using the interval between impulses. This creates uncertainties on the order of 5-10 parts per hundred. The overall performance of the nervous system is considerably better than 5 pph for many tasks. Thus the question arises: **What emergent property of a neural circuit overcomes the intrinsic noise limitations of the circuit's building blocks?** One answer is that the Law of Large Numbers applies to many (but not all) parallel neural circuits. So long as many neurons are each working on the same task in parallel, random noise in one can cancel that in another. This "averaging" of many individually-noisy results can yield low-noise performance, with scatter being reduced as the square root of the number of cells operating in parallel.

The major conditions for this "parallel precision principle" are: 1) the noise in each cell must be random and independent of that in another; 2) there must be a mechanism for summing together the results of these independent sources (e.g., the membrane potential of an integrating neuron downstream, or the tension in a muscle tendon in the case of motorneuron pools); and 3) if the number of inputs is to be varied, there must be a way of rescaling the sum according to the number of inputs used (for example, the input resistance of the summing cell may drop as more synaptic inputs become active, reducing all synaptic strengths).

I have previously discussed timing precision (J. Theoret. Biol. 104:121, 1983) but simple circuits can also be used for parameters other than time. Let binocular convergence angle (for vernier depth discrimination) be somehow encoded by noisy inputs in the membrane potential of a CNS cell. Let another neuron sum together the scaled output of **N** such cells; when its threshold is crossed, the uncertainty in the convergence angle it represents will be a factor $N^{-1/2}$ less than the original angular uncertainties. It would appear that most precision discriminations (e.g., orientation, hue, depth, elapsed time, pitch, etc.) would benefit from assigning many cells to the same task and "averaging" the results with such a parallel circuit. (Supported by NIH grant NS 04053).

218.12 THE CRAYFISH STRETCH RECEPTOR AS A MODEL OF SYNAPTIC INTERACTIONS BETWEEN PACEMAKER CELLS. O. Diez-Martínez, H. Quijano, J.A. Roig and J.P. Segundo. Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, México, D. F., 04510, MEXICO; Department of Anatomy, University of California at Los Angeles, Ca 90024, U.S.A.

We studied the isolated neuron of the crayfish slowly adapting stretch receptor organ (SAO) to test the applicability of a model that described the influence of a pacemaker cell upon another across a synapse with EPSPs (Segundo and Kohn, 1981). Since no excitatory fibers converge upon SAO, the EPSP effects were artificially induced by applying a brief stretch or "tug" to the receptor muscle. The tug shape, amplitude and duration were adjusted empirically so that its effect was that expected in a pacemaker neuron with excitatory monosynaptic input. SAO impulses were recorded extracellulary. A large number of tugs were applied irregulary, at relatively long intervals, and independently of the SAO discharge. In the perturbed interval the spike following the tug was advanced with respect to the expected or natural interval (N). The advance or "negative" delay was a V-shaped function of the phase (ϕ) i. e., the time elapsed between the last SAO impulse and the tug. The "delay function" was piece-wise linear with two segments. The vertex corresponded to the earliest tug arrival (λ) that triggered a SAO spike almost immediately. The parameter λ depended upon the tug characteristics. "Weak" or "strong" tugs resulted in a large or small λ, respectively. The left segment ($\phi<\lambda$) had a negative slope with some scatter around the best fitted-lines. The right segment ($\lambda<\phi<N$) had a positive slope near 1 and very little scatter. In 9/10 of cases the smallest ϕs had either no effect or slightly lengthened the perturbed interval. The fraction of the N where this occurred depended upon tug strength, increasing with λ. This behavior departed from the model predictions. Except for this anomalous effect with small ϕs, the SAO performance when subjected to tugs, mimicked a pacemaker neuron receiving excitatory monosynaptic input. Since preparations with such synaptic characteristics are scarce (i.e. Aplysia ganglion cells, Bryant et al, 1973) the artificially excited SAO provides an alternative with similar behavior, offering technical advantages for such studies.

219.1 VISININ (24Kd PROTEIN) AS A MARKER OF CONE CELLS IN VERTEBRATE RETINAE. N. Miki, S. Hatakenaka*, H. Kiyama*+ and M. Tohyama*+. Dept. of Pharmacol., Cancer Res. Inst., Kanazawa Univ., Kanazawa 920, Japan and+ Dept. of Neuroanat., Inst. Higher Nerv. Act., Osaka Univ., Osaka 530, Japan.

We have analyzed the soluble proteins of chick retina during development and found that a peptide of about 24,000 daltons (VISININ) appeared in the retina of the 14th day embryo and gradually increased in concentration with embryonic age until hatching. It was not detected in the cerebrum, tectum, pigment epithelium or vitreous body on SDS-PAGE analysis. It was partially purified by gel filtration and ion exchange column chromatography, and its isoelectric point was about 5.5. In the bovine retina, a protein was observed at 24,000 daltons on SDS-PAGE, but its isoelectric point was more basic than that of chick retina. Visinin was purified by extracting a corresponding band from SDS-PAGE and anti-visinin serum was produced by injecting it into a rabbit. The anti-serum showed a single precipitation line against purified visinin or partially purified visinin on either an Ouchterlony double immunodiffusion test or immunoelectrophoresis. Tissues were fixed with Zamboni's solution. The frozen sections were stained with indirect immunofluorescent staining (FITC) method. The photoreceptor cells were stained with anti-visinin serum from 7th day embryonic retinae and its intensity was gradually increased with embryonic age. Visinin-like immunoreactivity was also found in some kinds of amacrine and displaced amacrine cells from 11th day embryonic retinae. When human, cat, frog and carp retinae which contain both rods and cones were examined, staining of cone cells was clearly observed in the photoreceptor layer, but that of rod cells was not. Furthermore visinin-like immunoreactivity was barely detectable in the photoreceptor cells from bovine, rat and mouse retinae containing mostly rod cells. The results suggest that visinin is mainly located in the cone cells in various vertebrate retinae and provides a good marker for the cone cells, although the role of visinin in the cone cells is unknown.

219.2 MONOCLONAL ANTIBODIES SPECIFIC FOR GLIA IN THE DEVELOPING CHICK NERVOUS SYSTEM. V.P. Lemmon. Dept. of Anatomy and Cell Biology, Univ. of Pittsburgh, Sch. of Medicine, Pittsburgh, PA 15261.

Two monoclonal antibodies which bind to glia in the chick have been produced. Mice were immunized with primary cultures of retina enriched for "flat cells" which are thought to be derived from Muller cells. Hybridoma supernatants were screened immunohistochemically on sections of embryonic chick retina and optic tectum. Hybridomas producing antibodies that bound to Muller cells in the retina and radial glia in the optic tectum were cloned. EM-immunohistochemical and SDS-PAGE-TRANSBLOT analyses were conducted using two antibodies. Antibody 25-5E10 binds to an intracellular antigen with an apparent molecular weight of about 280KD. This antibody also binds to vascular endothelia and cartilage cells, but not to neurons. Antibody 25-3A7, an IgM, also binds to trans-blots under the conditions tested thus far. Antibody 3A7 does not bind to neurons or vascular endothelia. Tissue culture experiments have indicated that both of these antibodies bind to the cytoskeletons of "flat cells" prepared from embryonic chick retina, but not to neurons, and that the antigens are Triton-X100 insoluble.

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219.3 MONOCLONAL ANTIBODIES SPECIFIC FOR GANGLION CELLS IN THE DEVELOPING CHICK RETINA. C. Snyder*, J. Hailey*, and V. Lemmon (SPON: J.S. Lund). Dept. of Anatomy and Cell Biology, Univ. of Pittsburgh, Sch. of Medicine, Pittsburgh, PA 15261.

Twenty-four different monoclonal antibodies which bind specifically to ganglion cells in the embryonic chick retina have been produced. Mice were immunized with either: 1) optic nerves or 2) conconavalin-A binding proteins from embryonic day 14 (E14) chick retina. Hybridoma supernatants were screened immunohistochemically on sections of E14 retinas. Hybridomas which produced antibodies that bound to retinal ganglion cells or their processes were then cloned. The majority of these antibodies bind to axons of ganglion cells. Since these antibodies bind to axons very early in development (E6 earliest examined thus far), they should be useful in developmental studies of axon outgrowth and innervation of the optic tectum. The remaining antibodies bind to ganglion cell somas and their dendrites in the inner plexiform layer, as well as to axons. While EM-immunohistochemical studies have indicated that these antibodies bind to ganglion cell surface membranes, studies of live cells in tissue culture indicate that only a subset of the antibodies bind to cell surface molecules on retinal cells.

Supported by NIH grant # 7-R01-EY05285-01 to V. Lemmon.

219.4 PREGANGLIONIC NEURONS FROM THE EDINGER WESTPHAL NUCLEUS: GROWTH AND HISTOCHEMICAL CHARACTERIZATION IN CELL CULTURE. J.T. Fujii and D.K. Berg. Dept. of Biology, Univ. of California, San Diego; La Jolla, CA. 92093.

The preganglionic neurons that innervate the chick ciliary ganglion are located in the Edinger Westphal (EW) nucleus (a.k.a. accessory oculomotor nucleus) of the midbrain. Culture of these neurons would be valuable not only for examining cholinergic neurons of the central nervous system, but also for studying the interactions between EW and ciliary ganglion neurons in a defined environment in which both cell types are accessible. We report here that EW neurons survive in dissociated cell culture and express properties characteristic of their differentiated state in vivo.

Regions of the midbrain containing the EW nucleus were dissected from stage 40-41 chick embryos, dissociated with trypsin and trituration, and grown on a collagen substratum in Eagle's minimal essential medium with 10% horse serum and 5% chick embryo extract. Since many, although not all, EW neurons are known to contain leu-enkephalin-like immunoreactivity (ELI) and/or substance P-like immunoreactivity (SPLI) (Ericksen et al., 1982), as well as the conventional neurotransmitter acetylcholine, the cultures were tested for ELI, SPLI, and intracellular acetylcholinesterase using standard histochemical techniques. In each case labeled cells were identified. Cells positive for ELI were distinguished by labeled beaded processes, frequently associated with darkly labeled cell bodies. This pattern of labeling was completely eliminated by prior absorption of the primary antibody with leu-enkephalin. The number of cells labeled for ELI was equivalent to at least 20% of the number of cells that could be immunohistochmically stained in sister cultures for neurofilament protein, a marker for most classes of neurons. Fewer neurons were labeled for SPLI, numbering 10% or less of the ELI cells in sister cutures. Many, if not all, of the neurons in these cultures were positive when stained specifically for intracellular acetylcholinesterase, consistent with a cholinergic phenotype.

These results indicate that in dissociated cell culture EW neurons express appropriate charcteristics and that the proportion of these cells is sufficiently high to encourage study of their interactions with ciliary ganglion neurons. (Supported by NS 12601; JTF is an NRS Fellow.)

219.5 MONOCLONAL ANTIBODIES IDENTIFY NEURONS OF THE SOMATIC SENSORY AND MOTOR AREAS OF THE CEREBRAL CORTEX IN MONKEY. D.J. Schreyer and E.G. Jones, Dept. of Anat., Univ. of California, Irvine, CA 92717.

In order to uncover clues to the molecular diversity of the neurons comprising the cerebral cortex monoclonal antibodies (MCAs) were generated against cortical gray matter taken from the sensorimotor region of the monkey (M. fascicularis) using standard methods. These MCAs were screened by using them for immunohistochemical labelling of sections of monkey sensorimotor cortex in order to detect markers for antigens that are restricted to subsets of cortical neurons. Four such MCAs can be described: (1) 2E12 produces dense granular cytoplasmic labelling of the somata of a few large pyramidal neurons of layers III and V of the pre- and postcentral gyri. In addition, a larger population of cells are lightly labelled, predominately in layers III and V of somatic sensory and II and III of motor cortex, but with a few in other layers as well. Cellular labelling is accompanied by coarse labelling of the neuropil that is densest in more superficial layers. (2) 2F2 produces a dense labelling of cortical white matter and labelling of fine varicose processes in gray matter of sensorimotor cortex, and a smooth cytoplasmic labelling of a few small cortical cells. Processes are dense and disposed tangentially in layer I of pre- and postcentral areas, sparse and oriented radially and obliquely in layers II and III of both areas, and form a dense band in layers V-VI of motor cortex and in layer IV of somatic sensory cortex. Small cells can be seen in all layers, but are most common in I and II. (3) 3B11, like 2E12, labels large pyramidal cells of layers III and V, and also a few in layer II of motor cortex and VI of both areas. However, their is no population of less densely labelled cells, and coarse neuropil labelling is not seen. Cytoplasmic labelling of somata displays a smoother texture and often extends into proximal dendrites. (4) 3H4 produces a patchy surface labelling of a small number of medium to large neurons located in layers II-VI of motor cortex, but largely confined to deeper layers in somatic sensory cortex. The label appears to outline cells and proximal dendrites, and could reflect localizations of an antigen in presynaptic structures. An axonal localization of the antigen recognized by this MCA is also suggested by the accompanying dense labelling of the cortical white matter. Use of these MCAs on material from monkeys that received injections of the retrograde tracer Fast Blue in the spinal cord indicates that virtually all labelled corticospinal neurons are doubly labelled by 2E12,3B11 and 3H4, but not 2F2.

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219.6 CHEMICAL LABELS DEFINE OVERLAPPING SETS OF NEURONS IN THE LEECH CNS. G. Bablanian and B. Zipser. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

An extension of the classical approaches to the study of nervous system networks has been the application of immunological probes to define neurons into chemical sets. In the leech, we have characterized chemically labeled sets in the standard 400 neuron ganglion and the enlarged sex ganglia using 11 different antibodies. These antibodies are monoclonal antibodies generated against leech CNS. Drosophila CNS (Fujita et al., PNAS, 79:7929, 1982) and polyclonal antisera against FMRF-amide and serotonin. The antigenic sets specified by monoclonal or polyclonal antibodies range in size from 2 to 50 neurons and occur either as subsets of each other or as partially overlapping sets. Large overlapping sets stained by leech mab Laz2-1, Drosophila mabs 3A4, 8G1 and antisera FRMF-amide contain 30 to 50 functionally diverse neurons that overlap by 25 to 80%. Neurons in the intersection of several sets carry a combination of different markers. For example, the α cells, uniquely stained by Drosophila mab 2G4B, fall within all 4 larger antigenic sets. If large antigenic sets reflect neural networks or systems, then a neuron that is located in the intersection of several large sets becomes a multifunctional neuron. Overlapping antigenic sets specify combinatorial markers which may play a role in processes of synaptogenesis, synapse maintenance and ultimately the sculpting of neurons into pleiomorphic networks.

219.7 IDENTIFIED NEURONS IN THE BUCCAL GANGLIA OF HELIX POMATIA AND THEIR FUNCTIONAL SIGNIFICANCE. U. Altrup, E.-J. Speckmann and H. Caspers. (SPON:A.J.Berman) Physiol.Inst. 44 Münster F.R.Germany.

Functions of four visually identifiable giant neurons (B1 to B4) in the buccal ganglion of Helix pomatia have been studied using electrophysiological and morphological techniques. The investigations provide more precise insights into the function of this nervous system on a cellular level.

Action potentials (AP) of neuron B1 induce contractions of longitudinal muscle fibres of the oesophagus and stomach and enhance spontaneous peristaltic contractions. These effects are obviously mediated by thin terminal axonal fibres of the neuron situated in the wall of the oesophagus and stomach. The fibres show multiple swellings probably representing synaptic sites. Excitatory synaptic inputs to neuron B1 originate from an intraganglionic generator of pharyngeal feeding activity, from the cerebral ganglia and from peripheral neurons in the target regions of the buccal ganglia. Inhibitory inputs are evoked by unidentified neurons of the buccal ganglia. The inputs enable a coupling of the motor activities of the oesophagus/stomach to the pharyngeal activity during food uptake.

Terminal axonal fibres of neuron B2 are found in both salivary glands in the vicinity of epithelial cells. The fibres show multiple swellings. There are no findings suggesting a sensory or a direct motor function of the neuron. Neurons B2 in the left and right buccal ganglion are coupled electrically. The AP of both cells appear either synchronous or alternating or independent. The synaptic inputs to neuron B2 can increase the probability of occurrence of AP during food uptake. This effect possibly provides a coupling of the salivary glands to the pharyngeal activities.

The axons of neuron B3 can be followed through the cerebral ganglion to the kidney by means of antidromic stimulation. The neuron is mainly activated by unidentified neurons of the buccal ganglion.

Neuron B4 is a motoneuron of eleven pharyngeal muscles (Peters and Altrup, J. Neurophysiol. in press). It receives synaptic inputs from intra- and extraganglionic sources mainly during retraction of the radula. Neuron B4 is electrically coupled to unidentified synergistic motoneurons of the buccal ganglion.

220.1 MONOCLONAL ANTIBODY M6 BLOCKS NEURITE EXTENSION IN CULTURED MOUSE CEREBELLAR NEURONS. C. Lagenaur, S. Fushiki*, and M. Schachner. Dept. of Anatomy and Cell Biology, Univ. of Pittsburgh, Sch. of Medicine, Pittsburgh, PA 15261, Inst. of Neurobiology, Univ. of Heidelberg, Heidelberg, FRG.

A 35K dalton cell surface glycoprotein designated M6 has been detected in embryonic and adult mouse brain by a monoclonal antibody (anti-M6). Monoclonal anti-M6 at levels as low as 1 µg/ml has a striking inhibitory effect on neurite extension when included in the culture medium of early postnatal mouse cerebellar neurons. The total number of neurites is reduced and the few remaining are short and rough in appearance. Neuronal cell bodies exhibit some reduced viability after 2-4 days of antibody treatment as judged by dye exclusion, but astrocytes and oligodendrocytes are not effected. M6 can be detected first at embryonic day 10 on cells that have left the ventricular zone. Based on double immunofluorescence studies with cultured postnatal mouse cerebellar cells, M6 expression is almost exclusively neuronal. In retina, however, M6 appears to be additionally expressed on pigment epithelium and Müller cells. Immunoaffinity chromatography with deoxycholate solubilized brain membrane identifies M6 antigen as a protein that has an apparent molecular weight on SDS PAGE of 34-36K daltons. Molecular sieve chromatography of deoxycholate soluble antigen reveals an apparent molecular weight of greater than 8×10^6, indicating that M6 exists as large aggregates. Polyclonal rabbit antibodies against affinity purified M6 recognize a protein of similar molecular weight in many rodent species, human, chicken, and frog.

220.2 MONOCLONAL ANTIBODIES TO NEURAL MEMBRANES OF APLYSIA. D. P. Viele*, F. Strumwasser and K. D. Lovely*. Marine Biological Laboratory, Woods Hole, MA 02543.

Monoclonal antibodies (MAbs) against neural membrane antigens have proven to be valuable probes in the identification and mapping of antigenic markers expressed during neurogenesis. Recently MAbs have also been shown to be useful as reagents for specifically interferring with a variety of cellular functions. We have used neural membranes of Aplysia californica as immunogens, focusing on the peptidergic bag cells (BCs), which generate a cAMP-dependent pacemaker discharge, and the eye, which contains a circadian pacemaker system. MAbs which identify these systems would have the potential to interfere with pacemaker function and to reveal function-related antigens.

BCs (10-20 clusters) or eyes (10-20) dissected from adult Aplysia were homogenized in 2mM Tris/2mM EGTA/.02% PMSF (pH=8.0) buffer, centrifuged 10 min at 80,000 X g, and the crude membrane pellet resuspended in saline and emulsified with Freunds adjuvant. BALB/c mice received a series of 3 IP injections of either BC or eye membranes. Four days following the final injection, the spleens were removed, the spleenocytes fused with an Sp2 murine myeloma line and plated into 96 well plates. Surviving hybrids were screened against BC or eye membrane antigens using an immunodot test. Two fusions have produced 14 hybridoma lines secreting MAbs against BC membrane antigens and 28 lines secreting MAbs against eye cell membranes. A third fusion against abdominal ganglia (excluding BCs) is in progress.

Immunofluorescent staining of eye cells in primary culture revealed a diversity of staining patterns: some MAbs label photoreceptors exclusively, while others preferentially label various non-photoreceptor cells. Immunofluorescent staining of cultured BCs also resulted in different staining patterns: several MAbs stain only the cell membrane while others label the entire soma as well as neurites. Many MAbs recognize antigens common to neuronal (buccal ganglion, eye, BCs) as well as non-neuronal (buccal muscle, heart, gonad, atrial gland) membranes. Nine of the MAbs react with membranes isolated from mouse cerebral cortex, cerebellum or eye. Experiments to evaluate MAbs as pharmacological tools are in progress and involve intracellular pressure injection into BCs in primary culture.

220.3 CALCIUM ION ALTERS THE ASSOCIATION BETWEEN EXOGENOUS GANGLIOSIDE GM1 AND NEUROBLASTOMA CELLS IN CULTURE. K. C. Leskawa*, R. E. Erwin* and E. L. Hogan (SPON: N. Banik) Department of Neurology, The Medical University of South Carolina, Charleston, SC 29425.

Previously we have reported that, within a mixture of exogenous gangliosides, there may exist a combination of in vitro neuritogenic signals, that is, GM1 was most effective in promoting neurite extension, while trisialosyl gangliosides (GT1b) were most effective in promoting neurite sprouting and branching (Leskawa and Hogan, Soc. Neurosci., Boston, MA, 1983).

The present studies address the binding of exogenous GM1 to mouse neuroblastoma cells (N2A) in vitro. Three modes of association were defined: (1) a weakly hydrophobic form, where 3H-GM1 is labile to washing in a serum-containing medium; (2) a 'lectin-like' binding, which is labile to trypsin treatment; and (3) a mode incorporated into the plasma membrane lipid bilayer, which is stable to the above treatments (Facci et al., J. Neurochem., 42, 299, 1984).

Neuroblastoma cells in culture were synchronized in the G1/GO phase by serum deprivation for three days, and removed from the substratum. 3H-GM1, at 0.1 mM, was added to cells in suspension in the presence of varying concentrations of CaCl2 (0 to 25 mM) in HEPES buffered saline (HBS).

Calcium ion lowered all modes of 3H-GM1 binding to neuroblastoma cells. After 4 hr incubation, serum-labile GM1 was still not saturated and Ca++ concentrations, as low as 0.01 mM, inhibited this form of binding up to 50%. Ca++ at 0.01 and 0.1 mM (equimolar with respect to exogenous GM1) inhibited the trypsin-labile binding of GM1 to neuroblastoma approximately 50%. Higher concentrations (1 to 25 mM) resulted in even lower binding (30% of control). Similar data was obtained for GM1 incorporated into the lipid bilayer: 0.01 and 0.1 mM Ca++ lowered incorporation 60% and higher concentrations inhibited incorporation even further (80% compared to control).

Considering the documentation of ganglioside cross-bridging by Ca++, and that Ca++ renders gangliosides to be more hydrophobic in a biphasic partition system (Quarles and Folch-Pi, J. Neurochem, 12, 543, 1967), one would expect Ca++ to increase, rather than decrease, incorporation of exogenous GM1. These results suggest that interactions between divalent cations and exogenous gangliosides which affect incorporation into cellular membrane lipid bilayers cannot be fully explained by considerations of lipophilicity alone.

220.4 A POLYSPECIFIC ANTISERUM DISRUPTS NEURONAL-ASTROGLIAL INTERACTIONS IN POSTNATAL CEREBELLAR CULTURES. J.C.Edmondson and M.E.Hatten. Department of Pharmacology, New York University School of Medicine, New York, NY 10016.

In primary cerebellar monolayer microcultures from neonatal mice, glial filament protein positive (GFP+) astroglial cells grow extensive processes, and greater than 90 percent of the neurons are found within one cell diameter of an astroglial process after 24 hours in vitro (Hatten and Liem; JCB 90:622-630).

We describe here an antiserum which, when added to growing cultures as whole serum or IgG fraction, quantitatively inhibits astroglial process extension, and, in addition, when added as the Fab fraction, quantitatively inhibits formation of the close appositions between neurons and astroglial cells. In contrast, both the astroglial process outgrowth and the close appositions between astroglial cells and neurons appear normal in cultures grown in the presence of 1) the pre-immune serum, 2) an antiserum against the NILE-glycoprotein, a neuronal surface marker, or 3) an antiserum against BSP-2, an interneuronal adhesion molecule identical to N-CAM. The disruptive activity of our antiserum can be neutralized by preabsorption with dissociated cerebellar cells but not with PC12 cells, a neuron-like clonal line that does not form close associations with cerebellar astroglial cells in vitro. In tissue sections and in monolayer cultures from the cerebellum, the antiserum stains all cell surfaces and processes. It recognizes several bands in immunoblots against detergent-extracted cerebellar proteins, indicating that it is polyspecific.

These studies provide evidence that both 1) the in vitro astroglial morphological differentiation previously shown to be induced by the presence of neurons (Hatten; Neurosc. Abstracts 9:338, 1983) and 2) the close neuronal-astroglial appositions formed in vitro are mediated by neuronal-astroglial interaction molecules, or NAIMs, which can be blocked by antibodies. We have therefore named our disruptive antiserum, "anti-neuronal-astroglial interaction molecules," or "anti-NAIMS." We are currently using our culture system as a functional assay to purify the NAIMs.

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220.5 REGIONAL AND SUBCELLULAR DISTRIBUTION OF THY-1 IN HUMAN BRAIN. Per Almqvist*,Sven Carlsson*,William Wallace,and Bengt Winblad* (SPON:S.I.Walaas). Departments of Physiological Chemistry and Pathology,Univ. of Umea, S-901 87 Umea,Sweden and The Rockefeller Univ. New York,NY 10021.

The Thy-1 antigen is a membrane-bound glycoprotein that has been highly conserved during evolution. It exhibits variations in tissue distribution in different species. But in all species tested,it is present in high concentrations within the brain. However,the precise location and variation in concentration in the regions of the brain has not been determined. In order to begin these determinations,Thy-1 was purified from human brain cortical membranes and used to prepare monospecific rabbit antibodies.The antibodies were covalently coupled to an insoluble polymer (cellulose) to use for a highly precise radioimmunoassay. The detection limit of the assay was 1ng/ml. Radioimmunoassays of Thy-1 in homogenates of 12 brain regions showed that Thy-1 is present throughout the human brain. However,great variations were found in the expression of the glycoprotein between different regions. These regions could be divided into 3 groups by their Thy-1 content: 1. caudate nucleus,cerebral cortex,and putamen (2.5 ug Thy-1/mg protein); 2.globus pallidus,hippocampus,substantia nigra,thalamus,hypothalamus,and subcortical white matter (1.2-1.9 ug Thy-1/mg protein); 3. cerebellar cortex,pons,and medulla oblongata (0.5-0.7 ug Thy-1/mg protein). Thus,Thy-1 appears to be generally enriched in gray matter. Interestingly,the cortex of the cerebrum contains more of Thy-1 than that of the cerebellum. Radioimmunoassays of sub-cellular fractions from human frontal cortex prepared by sucrose gradient centrifugation, indicate the association of Thy-1 with synaptosomal plasma membranes. Moreover,Thy-1 was found to be enriched in the myelin fraction. However,the presence of Thy-1 on entrapped axonal plasma membranes is possible.In preliminary experiments to determine the subcellular location of Thy-1 synthesis,membrane-bound polysomes isolated from mouse brain and a mouse lymphocyte cell line (BW5147) were found to synthesize an antigen of Mr 20 kD in a cell-free translation assay. The synthesis of the antigen required the presence of dog pancreas microsomes indicating post-translational processing of the newly synthesized molecule.

220.6 ANALYSIS OF NEURONAL CELL SURFACE GLYCOPROTEINS USING FLOW CYTOMETRY AND FLUORESCENT LECTINS. James F. Leary and Mary F.D. Notter, Departments of Pathology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

The cell surface is thought to play the most important role in the control of embryonic growth, differentiation and morphogenesis in the nervous system. Biochemical analysis of synaptosomal preparations indicate that glycoproteins with their particular localization and potential for diversity may be mediators of recognition. We have initiated studies of the cell surface of a differentiating neuroblastoma cell line (N_2AB-1) as a model system utilizing the sensitive techique of flow cytometry-cell sorting and specific fluorescent membrane probes. Cells are differentiated by treatment in culture with prostaglandin E_1 (10 ug/ml) and dibutyl cyclic AMP (500 ug/ml) for a minimum of four days. Differentiated N_2AB-1 show neurites up to 45 um long and are arrested in Go_1G_1 of the cell cycle as determined by propidium iodide staining and flow cytometric analysis. Differentiated and actively growing cultures were treated in suspension with specific fluorescein labeled lectins including wheat germ agglutinin (FL-WGA) specific for A-acetylglucosamine, soybean agglutinin (FL-SBA) specific for N-acetylgalactosamine, concanavalin A (FL-ConA), specific for mannose residues, and ulex europeus agglutinin (FL-UEA) specific for fucose residues. Flow cytometric measurements of control and differentiated neuronal cells reacted with the various FL-lectins were made at the single cell level at rates of more than 3000 cells/second. Flow cytometric analysis revealed cell surface binding heterogeneity when FL-SBA and FL-ConA were reacted with the N_2AB-1 cell line. However, no differences in surface binding were seen between control and differentiated neuronal cells. When FL-UEA was reacted with control or differentiated neural cells, no binding was apparent indicating the lack of cell surface fucose on these cells. Furthermore, pretreatment of cells with neuraminidase before FL-UEA binding did not reveal masked fucosyl sites. When differentiated N_2AB-1 were exposed to FL-WGA, more cells bound more FL-WGA than that seen for control cultures. These data indicate that differentiation within the nervous system brings about a change in cell surface glycoproteins and that an increase in N-acetyl-glucosamine may be important in surface maturation of neurons.

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220.7 PC12 PHEOCHROMOCYTOMA CELLS SYNTHESIZE COMPLEX BRAIN GANGLIOSIDES. K.M. Walton and R.L. Schnaar. Depts. of Pharmacology and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Gangliosides are concentrated on nerve cell membranes and may be important in neuronal cell function. While complex trisialogangliosides are prevalent in brain, most homogeneous neuroblastoma cell lines contain only simple gangliosides with one or two sialic acids. Although preliminary ganglioside analyses have been performed on PC12 pheochromocytoma cells (which have been used in studies of neuronal differentiation and function), the chemical composition of their gangliosides have not been determined and the analyses are conflicting. Thus, we have initiated purification and chemical analysis of PC12 gangliosides.

Gangliosides were extracted from PC12 cells and separated according to the number of sialic acids (as determined using bovine brain ganglioside standards) on DEAE-Sepharose eluted with a salt gradient. Appoximately 1/5 of the sialic acid occurs as monosialoganglioside and 2/5 each as disialo- and trisialogangliosides. Two species eluting as trisialogangliosides were separated by silicic acid chromatography and further analyzed. Peak I co-chromatographed with standard ganglioside GT1b. Partial formic acid hydrolysis generated species which co-chromatographed with standard GD1b, GD1a, and GM1, suggesting that this PC12 ganglioside is GT1b. Mass spectral analysis by fast atom bombardment revealed a molecular ion (2211 amu) and a fragmentation pattern consistent with a trisialoganglioside. Partial formic acid hydrolysis of the slower moving Peak II generated species chromatographing with standard GD1b and GM1, suggesting that Peak II may be a different trisialoganglioside.

The ganglioside pattern of PC12 cells was analyzed as a function of differentiation with dexamethasone (10^{-5} M) or snake venom nerve growth factor (NGF, 2 ug/ml). While the predominant complex ganglioside concentrations were unchanged, there did appear to be a 3-fold (or higher) increase in the major high mobility (monosialoganglioside) peak in cells differentiated with NGF. Although the identity of this species is unknown, it is significantly less mobile than standard GM1 in two TLC systems.

Thus, it appears that the PC12 pheochromocytoma cell line contains a complex ganglioside complement comparable to nerve tissue which includes trisialogangliosides. In addition, the ganglioside pattern is selectively modified by the conditions of growth, and perhaps by cellular differentiation. Supp. by NIH grants HD14010 and GM07626.

220.8 IDENTIFICATION OF CEREBELLAR INHIBITORY INTERNEURONS WITH A MONOCLONAL ANTIBODY. J.M. Levine*, L.L. Beasley*, and W.B. Stallcup. (SPON: J.H.Steinbach). Molecular Neurobiology Laboratory, The Salk Institute, San Diego, CA 92138.

The NG2 antigen is a cell surface, chondroitin sulfate proteoglycan which was first identified with a rabbit antiserum raised against the B49 cell line (Cold Spring Harbor Symp., 48, p.761-774). We have identified and characterized cells expressing NG2-like immunoreactivity in the rat cerebellum.

Tissue sections of adult rat cerebellum were treated with monoclonal or polyclonal anti-NG2 followed by either a fluorescein or HRP labeled secondary antibody. Many cells throughout the molecular layer were labeled. Label was distributed in a ring-like fashion around cell bodies and along the major dendrites. These cells had the appearance of stellate and basket interneurons with processes which ramified throughout the molecular layer and at the level of the Purkinje cell bodies. Relatively few cells within the granule layer were labeled with the antibody; these NG2 positive cells had the appearance of small Golgi cells. The NG2 positive cells of the cerebellar cortex were not labeled with antibodies against the glial proteins GFAP and S100.

We examined the expression of the NG2 antigen by cerebellar cells in culture where it is possible to label individual cells with 2 different markers. When the cerebellar cells were grown in chemically defined medium (N2), between 50-60% of the NG2 positive cells bound tetanus toxin, a neuronal marker. In separate experiments, 45-50% of the NG2 positive cells contained neurofilament antigens but less than 3% had GFAP antigens. Inhibitory cerebellar neurons possess high affinity uptake systems for exogenous ^{3}H-GABA. Approximately 60% of the NG2 positive cells took up ^{3}H-GABA in a neuronal manner, i.e., uptake was inhibited by DABA but not by β-alanine. Thus, a significant number of the NG2 positive cells in the cultures have 3 properties associated with inhibitory interneurons. Expression of the antigen, however, was affected by media components. In cultures grown in DMEM containing 10% fetal calf serum and 20mM K^+, over 80% of the NG2 labeled cells were stained with anti-GFAP.

These findings demonstrate that the NG2 proteoglycan is a cell surface marker for cerebellar interneurons. Because the molecular heterogeneity of the surface of different populations of cerebellar cells is likely to be an important factor in morphogenesis, anti-NG2 antibodies may be useful for the analysis of cerebellar development.

220.9 DISTRIBUTION OF ACETYLCHOLINESTERASE AND α-BUNGAROTOXIN ON LONG-TERM DENERAVATED ADULT RAT MUSCLE FIBERS IN CULTURE IS UNAFFECTED BY THE PRESENCE OF EMBRYONIC SPINAL CORD EXPLANTS. J.J.Jay* & K.F. Barald (SPON: B. Oakley). Dept. Anatomy and Cell Biology, Univ. of Mich. Med. School, Ann Arbor, Mi. 48109.

The effect of long periods of denervation on properties of individual adult rat muscle fibers in culture was determined by examining: 1. the morphology and composition of the muscle fibers including the basal lamina, 2. the distribution of acetylcholinesterase (AChE), and 3. rhodamine α-bungarotoxin (RBTX) binding to putative acetylcholine receptors (AChR's). Flexor digitorum brevis (FDB) muscles were denervated 4 to 20 months prior to culture by cutting the sciatic nerve proximal to the thigh and directing it superiorly. These cultures were compared to normal muscle fibers cultured under the same conditions.

Long-term denervated fibers dissociated from the FDB have been kept in culture for 5 days. Prior to plating, the fibers exhibited discrete endplate-associated AChE and RBTX binding. After 24 hrs. in culture, although AChE remained at the neuromuscular junction, no RBTX binding was noted. AChE remained at the endplate only up to 48 hrs in vitro. Addition of rat embryonic ventral spinal cord (s.c.) explants had no effect on the presence or distribution of AChE or RBTX. Laminin, visualized with an affinity purified antibody provided by Dr. M. Wicha, was discontinuously distributed on the fibers before plating. Outgrowth of neurites to the fibers appeared non-directed.

Normal FDB fibers similarly cultured exhibit different characteristics. These fibers have been maintained for as long as 5 weeks in culture. Junctional AChE and colocalized RBTX are found on most of the fibers up to 48 hrs. in culture. The addition of s.c. explants maintained endplate AChE and AChR's (RBTX binding) through 96 hrs. In the absence of s.c. explants the majority of fibers exhibited extrajunctional AChE localization and no RBTX binding. Laminin distribution was uniform on fibers examined prior to plating. Neurite outgrowth in co-cultures was similar to that in long-term denervated co-cultures. Supported by a Rackham grant to J.J.J., by USPHS NS17017 and NS17262, the Muscular Dystrophy Association and Dysautonomia Foundation to K.F.B. We thank Drs. Bruce M. Carlson and Fay Hansen-Smith for providing us with denervated material and Dr. D.S. Grega for contribution to the initial experiments.

220.10 CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO RABRIT BRAIN ACETYLCHOLINESTERASE. S. Brimijoin and K.P. Mintz* (SPON: J.R. Daube). Dept. Pharmacology, Mayo Medical School, Rochester, MN 55905.

Eleven monoclonal antibodies (all IgG_1 or $IgG2_a$) were raised against rabbit brain acetylcholinesterase (AChE) purified to electrophoretic homogeneity (specific activity 2950 I.U./mg protein) by a two step procedure involving immunoaffinity chromatography. Binding of antigen was determined in an immunoadsorbance assay in which the activity of bound and free AChE was measured after exposure to antibody and precipitation by rabbit antimouse IgG complexed to protein A-bearing Staphylococcus aureus cells. The isolated monoclonal antibodies showed dissociation constants of 16 nM to 108 nM for the AChE in freshly prepared crude extracts of rabbit brain (calculations assumed one epitope per catalytic subunit of AChE). In every case, immunoblotting experiments revealed a drastic loss of affinity for SDS treated enzyme, suggesting that all antibodies were directed against conformational determinants. Species-specificities were determined by comparing the binding of AChE from brains of rabbit, rat, guinea pig, cat, and man. One antibody had an exclusive preference for rabbit AChE; others avidly bound the enzyme of guinea pig, cat, or man; one was able to bind rat AChE to a modest extent (apparent Kd 2×10^{-6} M). Each antibody showed equal apparent affinity for hydrophilic AChE (100,000 x g supernatant of rabbit brain extract prepared without detergent) and hydrophobic AChE (detergent extract of residual material). Competition analysis revealed numerous interactions pointing towards overlapping epitopes on the enzyme surface (e.g., a 60% mutual interference between antibodies 4-22 and 4-40). Other types of interaction suggested that certain antibodies caused significant conformational changes in the enzyme (antibody 4-40 completely blocked the binding of antibody 4-43, but antibody 4-43 had no effect on binding of antibody 4-40). The properties of antibody 3-43 were consistent with binding to the active site of rabbit AChE (50% inhibition of AChE activity at 10^{-8} M; 90% inhibition at 3×10^{-7} M; blockade of the binding of ^{3}H-DFP). This antibody and the others now in hand will be useful in further studies on the immunochemistry of mammalian AChE's. (Supported by NIH grant NS 11855.)

220.11 COLOCALIZATION OF ACETYLCHOLINE RECEPTORS AND BASAL LAMINA PROTEOGLYCAN AFTER DENERVATION. D.C. Linden, G. Pineda* and D. David* Biology Dept., Occidental College, Los Angeles, CA 90041

The quantitative distribution of a synaptic basal lamina proteoglycan (SBL-P) was correlated with that of acetylcholine receptors (AChR) in normal and denervated (3-9 weeks) Xenopus sartorius muscles. The heparan sulfate SBL-P (Anderson & Fambrough, 1983) was identified by a fluorescein labeled monoclonal antibody and was present predominantly in the basal lamina of the junctional folds. AChR were localized by rhodamine α-bungarotoxin (α-BGT) binding and quantified by measuring the total number of ^{125}I αBGT-receptor complexes.

Three areas of the muscle surface were studied: junctional (with junctional folds), intrajunctional (areas adjacent to the junctional folds within the junctional area), and extrajunctional (several hundred microns from any junction). SBL-P and AChR were colocalized in 98% of the contralateral and in 96% of the denervated junctional areas. Rarely were only AChR or only SBL-P associated with junctional folds.

In unoperated control muscles intrajunctional AChR and SBL-P formed a small fraction (0-20%) of the total staining on the muscle fiber. The majority of the intrajunctional staining was SBL-P only. Intrajunctional AChR and SBL-P were present after 3 weeks of denervation, and by 4-5 weeks increased to 20 times that of the control. At 8 weeks, intrajunctional labeling decreased to 6 times control. The changes in intrajunctional staining parallel first, the increase in ^{125}I αBGT-receptor complexes measured at 3 and 5 weeks, and then the decrease at 8 weeks post-denervation. At all times after denervation, the majority of intrajunctional staining was AChR colocalized with SBL-P (12-22 times the contralateral control). A slightly smaller amount (4-18 times the control) was SBL-P only. Much less frequently, receptors were present alone intrajunctionally (3-10 times control).

Extrajunctional accumulations of AChR and SBL-P were present in animals denervated longer than 5 weeks. A large amount of the extrajunctional staining was colocalized (40-60%). However, in some extrajunctional areas AChR or SBL-P occupied most, but not all of the staining area. In summary, the results of this research suggest that in most cases SBL-P and AChR at junctional, intrajunctional and extrajunctional areas of the muscle surface may be coregulated.

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220.12 BRAIN EXTRACT INDUCES REDISTRIBUTION OF BASAL LAMINA ANTIGENS. D.H. Feldman, J.R. Sanes, and J.C. Lawrence, Jr.*. Depts. of Physiology and Pharmacology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Embryonic rat myotubes assemble a basal lamina (BL) in culture. Small patches of this BL are stained by antibodies that bind selectively to the BL of the neuromuscular synaptic cleft in vivo. These synaptic BL (SBL)-like patches overlie clusters of acetylcholine receptors (AChRs). Saline extracts of rat brain (BE) induce formation of new SBL- and AChR-rich patches (Sanes et al., J. Neurosci. 4:464, 1984). Neural factor-induced AChR clustering is known to occur in the absence of protein synthesis (Christian et al., PNAS 75:4011, 1978), and to involve redistribution of AChRs within the plasma membrane (Salpeter et al., J. Cell Biol. 93:417, 1982). We therefore asked if BE-induced SBL patch formation also proceeds in the absence of protein synthesis and involves redistribution of BL components.

Cultured myotubes were fed medium containing BE and/or protein synthesis inhibitors (cycloheximide or puromycin at concentrations that blocked protein synthesis by 92-95%). One day later, patches rich in SBL antigens (detected with fluorescein-antibody) and AChRs (detected with rhodamine-bungarotoxin) were counted. BE increased the number of SBL- and AChR-rich patches 3-8 fold in the absence and 2-6 fold in the presence of inhibitors. The number of myotubes and the coincidence of SBL with AChRs were unchanged by BE and inhibitors.

To test whether SBL patches could form from extracellular antigens present prior to BE treatment, cultures were first incubated with antibody to SBL, then washed extensively. Control or BE medium was then added overnight, followed by staining with second antibody and bungarotoxin. Again, SBL- and AChR-rich patches coincided, and were increased 2.5-5 fold in BE treated cultures over controls. Thus BE induces extracellular BL components to form patches associated with clusters of AChRs. While BE may also stimulate synthesis and secretion of SBL components, our results suggest a mode of BL assembly in which previously externalized components can later be induced to redistribute and form specialized regions within the BL. (Supported by MDA and NIH.)

220.13 ADENYLATE CYCLASE OF CULTURES OF RAT SYMPATHETIC NEURONS AND STRIATUM: INFLUENCE OF VIP, SECRETIN AND DOPAMINE.
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Vasoactive intestinal peptide (VIP) and secretin (SE) were shown previously to stimulate tyrosine hydroxylase and adenylate cyclase in rat superior cervical ganglion (SCG) (Ip et al. Proc. Natl. Acad. Sci., USA, 79:7566, 1982) and certain cultured human neuroblastoma cells (Makman et al. Regulatory Peptides, 6:317, 1983). Both dopamine (DA) and VIP stimulate AC in striatum, and DA also stimulates AC in SCG of some species. We report here neurotransmitter regulation of AC in pure cultures of sympathetic neurons and in striatal cultures that exhibit neuronal properties including the presence of choline acetylase and substance P. Cultures of sympathetic neurons were prepared from dissociated SCG of 1 day old rats (Kessler et al. Science, 221:1059, 1983) and grown in serum-free medium. Cultures from dissociated striatum of rats at embryonic day 14-18 were grown either in serum-free medium or in medium containing fetal cell serum. AC of homogenates of sympathetic neuronal cultures was stimulated approximately 5 fold by 1 μM VIP or SE. At lower concentrations SE was slightly more potent than VIP, and glucagon, a structurally related peptide, was much less potent. At 10 μM, forskolin stimulated 4 fold, isoproterenol and histamine were inactive, and both N-ethyl-carboxamide adenosine (NECA) and DA produced small stimulations. In preliminary studies, growth of sympathetic neurons under depolarizing conditions (30 mM KCl) led to increased basal, forskolin and VIP/SE activity. AC of striatal culture homogenates was stimulated 7 fold by 10 μM VIP, and VIP was more potent than SE for AC activation. Striatal cultures also contained AC stimulated 2-3 fold by NECA, PGE, IPNE and DA. The DA stimulation was blocked by haloperidol but not by propranolol, consistent with the presence of D_1 receptors linked to AC in the striatal cultures. The presence and activity of these striatal receptor-ACs was dependent on the culture conditions used. It is concluded that cultures of fetal or neonatal sympathetic neurons and striatum express certain receptors coupled to AC that are also present in the adult. The cultures provide useful models for investigation of neuronal receptor-AC expression and regulation.
Supported by USPHS Grants NS-09649 and NS-20013 and by the American Heart Association and the Dysautonomia Foundation.

220.14 THREE MONOCLONAL ANTIBODIES FROM CHOLINERGIC SYNAPTOSOMES DEFINE RAT CNS NEURONAL SUBSETS.
P.D.Kushner and D.T.Stephenson* ALS and Neuromuscular Research Foundation, Pacific Medical Center, San Francisco, CA. 94115

We have identified three antibodies which demark groups or sets of neurons in the rat CNS. These sets are not the same but appear to be concentric. The monoclonal antibodies are from a collection generated against the Torpedo cholinergic synaptosome (Kushner, J.Neurochem,43,1984). This immunogen had the unique properties of being exclusively cholinergic and of deriving from one functional nucleus, which consists of homogeneous neurons that innervate the same target tissue, the electric organ.

The first of these, Tor 23, binds to the limiting membrane of very few neurons. In areas of the rat CNS we have studied, these neurons belong either to the motor or to the limbic system. The second antibody, Tor 103, binds the same neuronal population that is positive for Tor 23, but has a broader, less restrictive distribution. Tor 103 is localized to the inner plasma membrane and to filamentous structures within the neuronal cytoplasm. The third antibody, Tor 70, binds those neurons positive for Tor 23 and Tor 103, but has binding properties even more extensive. Tor 70 binding is localized to the inside of synaptic vesicles and to the outside of neuronal plasma membranes. Gel analysis reveals that the antigens defined by each of these antibodies are not the same. Tor 23 identifies two protein bands, 176 and 68 kd. Tor 103 identifies three protein bands, 190, 98, and 72 kd. Tor 70 binds to a large heterogeneous material at the top of the gel which has been identified as glycosaminoglycan (Carlson and Kelly, JBC 258: 1983).

Sets and subsets of membranal antigens may prove valuable in illucidating neuronal function in the mammalian CNS.

220.15 STUDIES ON THE TRANSMEMBRANE DISPOSITION OF THE NEURAL CELL ADHESION MOLECULE N-CAM.
G. Rougon*, G. Gennarini*, M. Hirn*, R. Sadoul*, H. Bazin* and C. Goridis*. (SPON: M. Schneider). Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, Marseille, France.

The N-CAM's are a group of surface glycoproteins involved in adhesive interactions between neurones. A monoclonal antibody reacting with a cytoplasmic domain of the molecule has been prepared. This antibody recognized the Mr 180,000 and the 140,000 proteins but not the Mr 120,000 chain which copurify from adult mouse brain. The latter polypeptide may lack a transmembrane fragment. Our conclusion that the N-CAM forms of higher Mr are transmembrane proteins was corroborated by our finding that they contain phosphoserine residues which can be labelled with (^{32}P)-phosphate in intact neuroblastoma cells. The transmembrane orientation of the polypeptide chains present in preparations of adult and neonatal mouse N-CAM was further studied using monoclonal antibodies and a model system to examine protease sensitivity after insertion into liposomes of N-CAM preparations. It is concluded that: a) identical polypeptide chains are present in young and adult preparations, b) the 180,000, 140,000 and 120,000 Mr chains differ by the length of their cytoplasmic extensions, and c) the longest cytoplasmic sequences have a Mr close to 90,000.

220.16 SPECIFIC ANTIBODIES TO TWO DIFFERENT Na,K-ATPASES.
K.J. Sweadner and R.C. Gilkeson*. Neurosurgical Research, Massachusetts General Hospital, Boston, MA 02114.

Mammalian brain contains two different molecular forms of the Na,K-ATPase. The two forms differ in their affinities for the cardiac glycosides and in their apparent molecular weight by gel electrophoresis. One form was purified from axolemma from myelinated axons of rat brain stem, and the other form from the renal medulla, a richer source than the brain. Rabbit antisera against each of the purified enzymes were characterized by staining of Western blots. Serum A2 contained antibodies specific for the axolemma Na,K-ATPase catalytic subunit, while serum K2 contained antibodies specific for the kidney Na,K-ATPase catalytic subunit. Two other sera, K1 and K3, contained antibodies which crossreacted with the catalytic subunits of both the axolemma and the kidney forms. Thus the two forms of the enzyme have some shared determinants and some which are distinct, which suggests that they have homology but significant structural differences as well.

The K2 and A2 antisera were used to screen a variety of tissues, including those with excitable, secretory, and absorbtive functions, for the presence of the two forms of the enzyme. The axolemma form (along with the kidney form) was found in large amounts only in the CNS, but it was detected in smaller amounts in several peripheral tissues. The kidney form predominates in peripheral tissues.

The K2 and A2 sera were also tested for their ability to detect two forms of the Na,K-ATPase in membranes from the brains of a variety of vertebrate species. The A2 serum was specific for the axolemma form in all mammals tested and in the frog. The K2 serum was specific for the kidney form only in rat, mouse, and hamster; in other mammals and the frog, it cross-reacted with both forms. Fish and the chicken showed only one form of the Na,K-ATPase by gel electrophoresis, but that one form contained determinants recognized by both A2 and K2 sera. The results suggest some interesting relationships in the evolution and divergence of the two Na,K-ATPases.

The sera contain crossreactive antibodies against the glycoprotein subunit of the Na,K-ATPase, but these could be removed by cross-absorbing K2 with axolemma and A2 with kidney membranes. The adsorbed sera were then used to stain rat glial and neuronal cell cultures. Thus specific reagents have been prepared for distinguishing the two forms of the Na,K-ATPase by immunocytochemistry.

220.17 FOUR MONOCLONAL ANTIBODIES BINDING TO RAT GLIOMAS. B.H. Liwnicz, M. Murphy*. Dept. of Pathology and Laboratory Medicine, University of Cincinnati Medical Center, Cincinnati, OH 45267

Our previous studies of gliomas in vitro showed an association between astrocytic differentiation and surface binding of lectins (Archer and Liwnicz, J. Neuropathol. Exp. Neurol. 42:343, 1983). The broad spectrum of lectin binding hampered our further studies on modulation of glioma surface determinants. We therefore chose an alternative approach and used monoclonal antibodies (MCAs) which are known to have a high degree of specificity.

Seven MCAs 10E7.9, 3G6.41, 3F4.18, 2B4.42, 4B7.29, B2C11, and B1F8 (to NG108 cell line or synaptosomal plasma membranes, R. Akeson, IDR, Cincinnati, OH) were screened against live cell suspensions of C6, RG2, and B82 rat glioma cell lines using indirect immunofluorescence (IIF) with FITC-labeled goat anti-mouse IgG. Four MCAs (B1F8, B2C11, 3G6.41 and 3F4.18) bound to the surface of all three gliomas with a uniform patchy distribution.

To evaluate the pattern of binding, seven rat glioma cell lines (RG2, C6, B82, 48A, T180A-2, T9 and TRF413-Cl 2) were cultured on coverslips in RPMI-1640 medium plus 10% fetal calf serum with or without 1 mM dibutyryl cyclic-AMP (dBcAMP), fixed with acetone and studied by IIF using the preselected MCAs. The rat glioma monolayer tissue cultures showed five distinctive cell types. Four of them (round cells [some showing mitotic figures], unipolar cells, bipolar cells and cells with multiple processes) bound all four MCAs. The fifth type, polygonal cells, was mostly negative. Treatment with dBcAMP enhanced the astrocytic differentiation but did not change the binding of MCAs to individual cell types.

Our study selected four MCAs to antigenic determinants of rat gliomas. The binding of MCAs is associated with cell types of the tumor.

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220.18 MONOCLONAL ANTIBODIES TO THE DEVELOPING RAT CEREBELLUM. M.S. Ghandour*, B. Foucaud* and G. Gombos. Centre de Neurochimie du CNRS, 5 rue Blaise Pascal, 67084 Strasbourg Cédex, France.

The developmental relationships between the cells in the CNS and the recognition of specific markers for each cell type in the nervous tissue are two major problems in developmental neurobiology. Molecules located at the cell surface and in the plasma membrane present a particular interest.

The present study describes a number of monoclonal antibodies obtained from the cell fusion of mouse spleen cells (immunized with 10-13 day old rat cerebellar particulate fraction) and P3-X63-Ag8 myeloma cell line. Balb/c mice received two subcutaneous injections and two intraperitoneal injections of 0.5 mg protein of the immunogene at three weeks intervals. The specificity of the monoclonal antibodies produced by each hybridoma was determined by screening the hybridoma supernatants for their differential binding to the particulate fraction from 10-13 day old rat cerebella and by immunocytochemistry on the 10-13 day old rat cerebellar sections. Nine different monoclonal antibodies are presented in the present paper.

Using immunocytochemistry, four monoclonal antibodies called MAbs H9, H10, H11 and H12 stained only neurones and neuronal structures : the MAb H9 labels the molecular layer, granule cells, glomeruli and the external granular layer. The MAb H10 stained all the neuronal cell perikarya. The MAbs H11 and H12 stained the molecular layer, granule cells and glomeruli. In addition, the Purkinje cell perikaryal surface was specifically stained with MAb H12. Preliminary results for MAbs H11 showed a preferential accumulation of the corresponding antigens at early stages of cerebellar development. On Western blots the antibody recognized two bands of molecular weight 185 and 120 KD from the membrane fraction.

Glial cells in cerebellum were stained by five different monoclonal antibodies called MAbs H8, H14, H15, H19 and H36. The staining pattern of glial cells obtained with each MAb was different from those obtained with any other. Preliminary results for MAb H8 showed a transient accumulation of the corresponding antigen during the first and second week after birth while with MAb 36 astrocytes were more intensely stained during the first week after birth than the adults. The biochemical characterization of the corresponding antigens and their ultrastructural localisation are now in progress.

220.19 FIBRONECTIN DISTRIBUTION ALONG NEURAL CREST PATHWAYS DURING XENOPUS LAEVIS DEVELOPMENT. D. Krotoski and M. Bronner-Fraser. Developmental Biology Center and Department of Physiology and Biophysics, University of California, Irvine CA. 92717

Fibronectin (FN) has been proposed to have important function in cell adhesion and as a substrate for migrating cells (Hynes and Yamada J.Cell Biol. 95:369,1982). FN has been visualized along neural crest pathways in the developing chicken embryo during active neural crest cell migration (Newgreen and Thiery, Cell Tiss.Res. 211:269,1980; Mayer, et al.,Dev. Biol 82:267,1981).

Here, we report the distribution of fibronectin along neural pathways in Xenopus laevis as a first step in defining mechanisms which control neural crest migration in this species. Xenopus embryos are easily manipulable and, therefore, provide a convenient model for the analysis of vertebrate neural patterning. Previous investigators have shown that FN is present in Xenopus along the roof of the blastocoel during gastrulation where invaginating mesodermal cells migrate (Lee et al. Cell 36:729,1984) and along the dorsal mesentery on which primordial germ cells move (Heasman et al. Cell 27:437,1981).

We have used an affinity purified anti-Xenopus fibronectin (generously provided by Drs. R. Hynes and G. Lee) to stain frozen sections. Unfixed Xenopus embyros were quick frozen on liquid nitrogen and cryostat sections were cut at 10 microns. The tissue was stained with antibody to fibronectin followed by a second rhodamine-conjugated anti-rabbit IgG. Embryos from three stages of development were observed: just prior to the onset of neural crest migration, during active migration, and at the cessation of migration. In early embryos, FN was present between the three germ layers, as reported by Lee, et al. (1984). Embryos processed at the time of neural crest migration (St. 26) exhibited fibronectin staining along both the dorsal and ventral neural crest pathways. Fibrils of FN were present around the somites, ectoderm, neural tube and notochord. Even at later Stages (e.g. St. 39), staining was pronounced under the ectoderm along the neural tube, notochord and mesentery. In addition, a thin band of FN was visualized between the dermamyotome and sclerotome after lamination of the somite. At all stages examined, the highest signal was present around the neural tube and notochord. FN was also present in the amphibian dorsal fin which is derived from neural crest cells. The fibronectin staining pattern we observed is grossly similar to that observed in other species.

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220.20 DORSAL ROOT GANGLION x NEUROBLASTOMA CELL HYBRIDS EXPRESS SENSORY NEURON SURFACE ANTIGENS. T.M.Jessell, J.Vovvodic*, W.D.Matthew and J.Dodd. Dept. of Neurobiology, Harvard Medical School, Boston MA 02115.

The identification of surface molecules on functional subsets of dorsal root ganglion (DRG) neurons has been hindered by the limited number and heterogeneity of cell types within DRG. We used rat DRG cells and the murine neuroblastoma line N18TG2 to generate somatic cell hybrids which exhibit many neuronal properties including surface carbohydrate antigens expressed by subsets of DRG neurons.

DRG cells were dissociated from 17d embryonic rats, preplated to remove non-neuronal cells, and fused with N18TG2 cells using PEG. Hybrids were selected in a medium of L15-CO_2 supplemented with 5% rat serum, NGF and HAT. After 2-3 weeks in culture, small foci of cells with phase bright somata were observed. Clonal lines were generated by expansion of single cells removed from different foci by micromanipulation. The properties of one line, E3H5-B1, have been characterized.

E3H5-B1 cells, induced to differentiate by growth in serum-free L15-CO_2 medium supplemented with transferrin, insulin, NGF, retinoic acid and dbcAMP exhibited phase bright cell bodies and long processes. E3H5-B1 cells expressed both Thy 1.1 and Thy 1.2 alloantigens and the cell surface glycoprotein coded by the $H2K^K$ locus of the mouse H2 complex, indicating the hybrid nature of these cells. Differentiated E3H5-B1 cells also expressed the A2B5 antigen, tetanus toxin binding sites and the 200kd neurofilament protein. The A2B5 antigen was not expressed by N18TG2 cells differentiated under identical conditions. DRG neurons and E3H5-B1, but not N18TG2 cells also bound monoclonal antibody 38/D7 which recognizes an antigen on sensory and autonomic peripheral neurons in the rat (Vulliamy et al Nature 291,418,1981).

The carbohydrate epitopes, SSEA-3 and SSEA-4, that identify subpopulations of DRG neurons, were present on E3H5-B1 cells. However, SNAC antigens (Dodd & Jessell, this volume) that delineate a separate population of DRG neurons were not expressed. The E3H5-B1 line, therefore, expresses surface antigens that characterize a subset of DRG neurons and may be useful for examining other surface properties of this class of neurons.

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221.1 GLUCOCORTICOIDS INHIBIT PROLIFERATION OF ASTROCYTES IN VITRO. Douglas A. Kniss and Richard W. Burry, Department of Anatomy and Neuroscience Research Laboratory, The Ohio State University, Columbus, Ohio 43210.

One response of the CNS to traumatic injury is a marked increase in the proliferative activity of the glial cells (especially astrocytes). This trauma-induced proliferation may be responsible for the formation of the glial scar at the lesion site. Steroid hormones have been shown to possess cell cycle regulating activity in several cell types. Thus, we used a primary glial cell culture system to examine the control of proliferative activity in glial cells by steroid hormones.

Glial-enriched cultures of 2-day old rat cerebellum were established in Ham's F12 medium supplemented with 10% FCS and 3 mM KCl.

The first experiment tested several steroids for their ability to inhibit cell cycling. After 12 days in vitro cultures were switched to chemically defined medium (CDM) (Kniss and Burry, Soc. Neurosci. Abst. 9:242, 1983) with 2% FCS added to some cultures as a mitogen. Steroids (corticosterone, dexamethasone, hydrocortisone, 17B estradiol, progesterone, testosterone, or aldosterone) were added to cultures at 10^{-7}M. Control cultures received an equivalent volume of 3% ethanol vehicle. One day later cultures were labeled with 5 uCi/ml ^{3}H-thymidine (^{3}H-TdR) for 24h. After incubation cells were treated with 10% TCA and aliquots of the resuspended pellets counted by liquid scintillation. Only the glucocorticoids, dexamethasone and corticosterone, inhibited DNA synthesis by glial cells.

The dose-response behavior of the most effective hormone, dexamethasone, was tested by adding the hormone (10^{-6} - 10^{-12}M) to 12-day old cultures in CDM and 2% FCS, followed 24 hr. later by labeling with ^{3}H-TdR. TCA-insoluble radioactivity measured 24 hr. later showed that 10^{-6} and 10^{-7}M dexamethasone inhibited DNA synthesis by 55.9 and 54.5%, respectively. Concentrations of 10^{-8} - 10^{-12}M showed no significant inhibition of DNA synthesis.

Finally, the kinetics of hormone-induced inhibition of glial cell proliferation was examined by treating cells with dexamethasone for times from 5 min. to 12h. A 5-min. exposure to the hormone was sufficient to cause a 51% reduction in DNA synthesis.

The results suggest that glucocorticoids are effective at controlling proliferation of cultured glial cells after only a 5-min. exposure. Supported by NIH Grant NS-19961 (Richard W. Burry) and the Spinal Cord Injury Research Center, NIH Grant NS-10165 (Richard W. Burry).

221.2 AXOLEMMA AND MYELIN-ENRICHED FRACTIONS PRODUCE DIFFERENTIAL MITOGENIC RESPONSES IN CULTURED SCHWANN CELLS. J.E. Yoshino*, M.P. Dinneen*, B.L. Lewis* and G.H. DeVries. Department of Biochemistry, Medical College of Virginia, Richmond, Virginia 23298.

Schwann cells proliferate during development and Wallerian degeneration. The axon appears to be responsible for the developmental response, whereas, breakdown of myelin seems to trigger proliferation during Wallerian degeneration. The axonal mitogen has been investigated previously using cultured neurites and axolemma-enriched fractions from CNS and PNS. We have found that both axolemma and myelin enriched fractions prepared from rat brainstem stimulate [^{3}H]thymidine uptake by cultured Schwann cells. The mitogenic activities of the two fractions can be differentiated by their dose response curves and the rates at which the label was incorporated.

Schwann cells were prepared from 2d rat sciatic nerves and were purified by treatment with anti-Thy 1.1. Axolemma and myelin-enriched fractions were prepared as described previously. After the Schwann cells had been incubated with axolemma or myelin for 24 hr, 0.3uCi of [^{3}H]thymidine was added to the cultures. Forty eight hr later the cells were collected using a Titertek cell harvester or processed for radioautography.

Increasing the concentration of axolemma produced a sigmoid dose dependent uptake of [^{3}H]thymidine. The shape of the dose response curve obtained with the myelin fraction resembled a rectangular hyperbola. Although the myelin membranes were a more potent stimulus at lower concentrations, the maximal response elicited by myelin was one-half that observed with axolemma. Similar results were obtained when the Schwann cells were examined by radioautography.

Schwann cells were harvested 12 hr, 24 hr, or 48 hr after the addition of [^{3}H]thymidine in order to examine the rate of incorporation. Axolemma treated Schwann cells exhibited little change in the rate of [^{3}H]thymidine uptake during these periods. Schwann cells incubated with myelin accumulated label three times faster during the 24-48 hr period following the addition of [^{3}H]thymidine when compared to the 12-24 hr interval. We conclude that cultured Schwann cells are capable of responding to two types of mitogenic membranes derived from fractionated nervous tissue: one of axolemmal origin and the second from myelin-related membranes. Supported by NIH NS-06819, NS-10821, and NS-15408.

221.3 SCHWANN CELL TRANSITION FROM A MYELIN MAINTAINING STATE TO A QUIESCENT STATE AFTER PERMANENT NERVE TRANSECTION Joseph F. Poduslo, Peter J. Dyck, and Carole T. Berg.* Membrane Biochemistry Lab, Peripheral Nerve Center, Neurology and Biochemistry, Mayo Foundation, Rochester, MN 55905

Permanent nerve transection of the adult rat sciatic nerve forces Schwann cells in the distal nerve segment from a myelin maintaining to a quiescent state. This transition was followed by serial morphometric evaluation of the percentage fascicular area having myelin (myelin % of area) in transverse sections of the distal nerve segment and revealed a rapid decline from a normal value of 36.6% to 3.2% by 14 days for the sciatic nerve to <1.0% throughout the remaining time course (up to 105 days). No evidence of axonal reentry into the distal nerve segment or new myelin formation was observed at times less than 70 days. In some of the distal nerve segments at 70, 90, and 105 days, new myelinated fibers were observed which usually consisted of only a few myelinated fibers at the periphery and in the worse case amounted to 1.6%. Radioactive precursor incorporation of [^{3}H]Man into endoneurial slices at 4 and 7 days after transection revealed two species of the major myelin glycoprotein, P_0, with M_r of 28,500 and 27,700. By 14 days after nerve transection, only the 27,700 species remained. Incorporation of [^{3}H]Man into the 27,700 species increased progressively to 35 days after transection and then began to decline at 70 and 105 days. Alterations in the oligosaccharide structure of this down regulated myelin glycoprotein accounted for the M_r shift and the progressive increase in mannose incorporation. Lectin affinity chromatography of pronase digested P_0 glycopeptides on Con A Sepharose revealed that the 28,500 species of P_0 had the complex-type oligosaccharide as the predominant structure (92%). In contrast, the high mannose-type oligosaccharide was the predominate structure for the 27,700 M_r form, which increased to 70% of the total radioactivity by 35 days after nerve transection. This 27,700 species of [^{3}H]Man labelled glycoprotein at 35 days after transection shifted to 28,500 with pulse chase, and this M_r shift corresponded to the transition between the high mannose-type and the complex-type oligosaccharide. It is concluded that this high mannose-type oligosaccharide is not an end product since additional processing can be demonstrated. The mechanism of down regulation in the biosynthesis of this major myelin glycoprotein, therefore, occurs gradually between 7 and 14 days after nerve transection with a biosynthetic switch from the complex-type oligosaccharide structure as an end product to the predominantly high mannose-type oligosaccharide structure as a biosynthetic intermediate.

221.4 ALTERATIONS IN ANEROBIC VS. AEROBIC METABOLISM IN INJURED CNS AND PNS NERVE. M. J. Politis. Dept. of Anatomy, Univ. of Saskatchewan, Saskatoon, SK Canada S7N OWO.

Periaxonal cells distal to site of nerve fiber injury undergo reactive changes subsequent to axonal degeneration. In the present study, alterations in activities of enzymes associated with anerobic (lactic dehydrogenase, LDH) vs. aerobic (succinic dehydrogenase, SDH) were assessed in traumatized rat central (optic) and peripheral (sciatic) nerve trunks.

Right optic nerves were crushed and right sciatic trunks transected. At 7 and 14 days postoperatively (d.p.o.) tissue distal to site of injury, as well as contralateral (control) nerve was removed and assayed for LDH and SDH activity.

Specific activity of LDH in traumatized peripheral nerves was 3 and 1.3 times that in contralateral control nerve tissue at 7 and 14 d.p.o., respectively. Elevations in LDH in crushed (vs. control) optic nerves were similarly observed, but were less dramatic (i.e., 1.8 fold gradient at 7 d.p.o. with no differences between crushed and control nerves at 14 d.p.o.).

SDH activity was similar in traumatized vs. unoperated nerves for both optic and sciatic nerves at 7 d.p.o. In contrast to results obtained for LDH, SDH activity in traumatized sciatic nerves was 3-fold lower than in contralateral control nerve at 14 d.p.o. Specific activity in crushed optic nerves was similar to that of control at this time point.

Results suggest an overall shift from aerobic to anerobic metabolism distal to site of injury in mammalian central and peripheral nerve tissue, the extent and duration of which is more pronounced in peripheral nerve. Experiments are in progress to determine if this shift is sensitive to administration of mitotic inhibitors.

221.5 PURIFICATION AND CHARACTERIZATION OF CULTURES OF OLIGODENDROGLIA FROM RAT BRAIN, S. E. Poduslo, K. Miller*, R. Curbeam*, and P. Reier*. Dept. Neurology, Johns Hopkins Univ. Sch. of Med. and Dept. Anatomy, Univ. of Maryland Sch. of Med., Baltimore, MD 21205.

By manipulation of culture conditions, it is possible to purify small dark cells from rat brain which by morphological criteria are oligodendroglia. The method, a modification of that of McCarthy and DeVellis (J. Cell Biol., 85, 890, 1980), involves plating dissociated cells from one- to three-day-old rat brain in Dulbecco's medium with 10% fetal calf serum. After a week in culture, a monolayer of flat cells with an upper layer of small dark cells is obtained. The small dark cells can be purified by shaking the cultures and replating the cells that have detached from the monolayer, using medium with 5% fetal calf serum. In this system, the small dark cells can be harvested several times from the parent flasks. It is necessary to constantly manipulate the cultures to retain homogeneous populations of small dark cells. Electron microscopy reveals that the small dark cells are differentiated oligodendroglia having a heterochromatic nucleus and a cytoplasm rich in organelles with prominent microtubules. Immunofluorescence staining of the small dark cells shows that only a small proportion are positive for cerebrosides (Rapport et al, J. Neurochem. 14, 9, 1967) or 04 antigen (Sommer & Schachner, Devel. Biol., 83, 311, 1981), markers for oligodendroglia; the small dark cells are negative for glial fibrillary acidic protein (Bignami, Eng, et al, Brain Res. 43, 429, 1972) and fibronectin. It is possible to increase the number of 04 positive cells by the addition of dibutyryl cyclic AMP to the medium. Increasing the serum concentration to more than 5% increases the number of astrocytes (glial fibrillary acidic protein positive cells) in the cultures. Incorporation studies showed that the small dark cells were able to incorporate [^{3}H]galactose into cerebrosides; this is a good biochemical marker for bulk-isolated oligodendroglia. Other radiolabeled substrates have also been analyzed. Thus a homogeneous population of small dark cells can be purified from rat brain which by electron microscopy and by incorporation studies can be identified as oligodendroglia. Expression of known glial antigens, however, is at a low level. Possibly once the cells are induced to produce myelin, the expression of these glial antigens will increase dramatically. Still it is now possible to purify potential myelin-forming cells from small animals. Supported by NIH-NS-14577 and 16956, the Multiple Sclerosis Society, and NIH-NS-13836.

221.6 EFFECTS OF PUTATIVE NOCICEPTIVE NEURO-PEPTIDES ON TAURINE RELEASE FROM GLIA. W. Shain, J.A. Greenhouse*, V. Madelian*, M. Perrone*, R. LaPore* (Spon: W.R. Cumisky). Lab. Neurotox. and Neurol. Disorders, Center for Labs. and Res., Albany, NY 12201 and Sterling Winthrop Res. Inst., Rensselaer, NY 12144.

LRM55 glial cells have substance-P (SP) receptors similar to those found in brain (Perrone et al. J. CELL BIOL. 97:931. 1983). Since stimulation of beta-adrenergic receptors in primary cultures of astrocytes and LRM55 glial cells results in release of taurine (Shain and Martin, NATURE, submitted), we have studied the effects of SP and activators of other neuropeptide receptors on taurine release. By itself SP has little or no effect on taurine release; however, when cells are coincubated with SP and the beta-adrenergic agonist isoproterenol (IPR) there is a dose-dependent inhibition of IPR stimulated release by SP (IC-50=7x10-10 M). In examining the effects of other peptide receptor agonists on taurine release in LRM55 glial cells, we have observed that delta- and mu-opiate selective compounds (DADDLE and RX78-3030) had no effect; however, the kappa selective compound U50488 did stimulate taurine release. The maximal amount of stimulation was similar to that observed with IPR. The cellular response to U50488 rapidly desensitizes and appears to have a slow rate of recovery. In order to construct accurate dose-response curves with U50488 it was necessary to expose cells to a single concentration of drug at a time. Normalization of the U50488 response to a standard IPR response was used to construct the dose-response curve (EC-50=4x10-11 M). Several mechanisms may be responsible for taurine release. Inhibition of taurine uptake is one possible mechanism, but neither compound was observed to effect taurine uptake. IPR stimulation of taurine release may be dependent on 3',5' adenosine monohosphate (cAMP). The effects of SP and U50488 on intracellular cAMP were examined. Neither compound significantly increased basal or IPR stimulated levels of cAMP. Thus, SP inhibits taurine release while U50488 stimulates release. However, since neither SP nor U50488 significantly stimulate cAMP accumulation, the mechanism(s) by which these compounds regulate taurine release appears to differ from beta-receptor regulation.

221.7 REGULATION OF TAURINE RELEASE FROM GLIA. V. Madelian*, D.L. Martin, W. Shain, R. Lepore*, M. Perrone*, Lab. Neurotox. Neurol. Disorders, Center for Labs and Res., Albany, NY 12201 and Sterling Winthrop Res. Inst., Rensselaer, NY 12144.

Activation of beta adrenergic receptors stimulate the release of the neuroactive amino acid taurine from primary cultures of astrocytes and LRM55 glial cells (Shain and Martin, Nature, submitted). Since beta-receptor activation results in stimulation of adenylate cyclase, the role of 3,5'-adenosine monophosphate (cAMP) as an intermediate in the process leading from receptor occupancy to taurine release was explored using three experimental approaches. First, the isoproterenol (IPR) dose dependencies of taurine release and cAMP accumulation were determined and shown to increase in parallel (EC50 for taurine release=5nM, EC50 for cAMP accumulation=20nM). Second, the non-hydrolyzable cAMP analogs dibutyryl- and 8-bromo-cAMP were used as potential stimulators and shown to elicit taurine release. Third, the time courses for taurine release and cAMP accumulation were determined and shown to rise rapidly to maxima about 5 min after exposure to agonist. These data are consistent with the hypothesis that beta-receptor activation mediates taurine release via adenylate cyclase activation.

Continued exposure of cells to IPR causes a decline both in cAMP accumulation and in taurine release rates. However, unlike their striking similarity during stimulation, the time courses during decline of these processes show significant differences. While cAMP levels drop continuously over 30 min ($t_{1/2}$ = 10 min) and approach initial levels, inactivation of taurine release occurs more rapidly ($t_{1/2}$ = 4.5 min) and reaches a new steady-state well above the background. In order to establish the possible role of cAMP depletion in the decline of taurine release, cells were exposed for 30 min to the non-degradable cAMP analog dibutyryl-cAMP. Cells thus treated showed an inactivation of taurine release similar to that observed with IPR. These latter experiments suggest that unlike the initiation of taurine release, the inactivation of release may be independent of cAMP.

221.8 CONVERSION OF GLUTAMATE TO GLUTAMINE BY RAT CORTICAL ASTROCYTES. D.L. Martin, R.A. Waniewski, and H.S. Miller*. Ctr. for Labs & Res., NYS Dept. of Health, Albany, NY 12201.

Brain metabolism studies with radiolabeled precursors provided the first indication that glutamate and glutamine are metabolized in different compartments in the nervous system. The glutamine cycle has been proposed as a mechanism whereby glutamate released from neurons is salvaged by glial cells, converted to glutamine and returned to neurons. A key enzyme in this pathway, glutamine synthetase, has been localized exclusively within astrocytes and its in vitro activity is high. However, studies of the activity of this enzyme and the operation of the glutamine cycle in intact astrocytes and C6 glioma cells suggest that the conversion of glutamate to glutamine is a rather minor metabolic pathway.

We have examined the time course of ^{14}C(U) glutamate metabolism by astrocytes and identified the products by using HPLC coupled with fluorescence detection and scintillation counting. Primary rat cortical astrocytes cultured in 24 well trays were provided by Dr. Harold K. Kimelberg. Cells were incubated for various times from 1-120 min in HEPES-buffered Hank's medium containing 17 µM ^{14}C(U)-L-glutamate. The incubation medium (IM) was collected, cells were extracted with perchloric acid (PCA) and solubilized with NaOH. Of the original ^{14}C label added, 27% remained as glutamate after 2 hours, with 20% in the PCA extract and 7.4% in the medium. 41% of the label was recovered as glutamine, with 9.4% in the PCA extract and 31.6% in the medium. 15.6% of the label was found in a fraction not derivatized by OPT nor retained by the column. This fraction probably represents deaminated glutamate, intermediates of the TCA cycle. Finally 9.2% of the label was found in the PCA precipitate and may represent incorporation into protein. No significant amounts of label were recovered in the aspartate peak. Glutamate in the IM fell from 84 nmol/mg (relative specific activity(RSA)=1) at time 0 to 12 nmol/mg at 120 min (RSA=.55). Glutamate levels in the PCA extract fell from 52 ± 6 nmol/mg protein at 0 min to 26 ± 2 nmol at 120 min (RSA=.55). Glutamate levels in the PCA extract rose from 7.2 ± 2.5 nmol/mg (RSA=0) at time 0 to 14.2 ± 2.9 nmol/mg (RSA=.25). Glutamine in the IM rose from 0 to 98 ± 23 nmol/mg protein at 120 min (RSA= 0.25). These results indicate that the glutamine cycle may be the major pathway of glutamate metabolism under these conditions.

221.9 HIGH AFFINITY UPTAKE OF [^{3}H]SEROTONIN BY PRIMARY ASTROCYTE CULTURES FROM RAT BRAIN. H.K. Kimelberg and D.M. Katz*. Div. of Neurosurgery, Albany Medical College, Albany, N.Y.

Primary astrocyte cultures prepared from the cerebral cortices of neonatal rats show significant accumulation of [^{3}H] serotonin ([^{3}H]5HT). At concentrations in the range of 0.01 to 1.0μM [^{3}H]5HT, this uptake was 70-80% Na^+ dependent. Kinetic analysis revealed that the Na^+ dependent component had a K_m of 0.40 ± 0.11μM (± S.E.M.) [^{3}H]5HT and a Vmax of 1.61 pmoles [^{3}H]5HT/mg protein/min. In the absence of Na^+ the uptake was non-saturable. Omission of the monoamine oxidase inhibitor pargyline markedly reduced the Na^+ dependent component of ^{3}H5HT (10^{-7}M) uptake, but had a much smaller effect on the lower Na^+ independent component. This suggests that there is significant oxidative deamination of the 5HT taken up. After a 60 minute exposure to 10^{-7}M [^{3}H] 5HT the cells accumulated around 20 pmoles/mg protein, and this accumulation was usually still continuing. Based on an average intracellular volume of 4μl/mg protein this represented an estimated intracellular concentration of 5 x 10^{-6}M 5HT, or a cell to medium concentration ratio of about 50. Inhibition of [^{3}H]5HT uptake by selective monoamine uptake antagonists was also consistent with a specific high affinity uptake mechanism for 5HT. Thus, the order of effectiveness of inhibition was chlorimipramine > imipramine > fluoxetine = amitriptyline > desmethylimipramine > mianserin = iprindole. The approximate IC_{50} values obtained from inspection of the dose response curves were 0.025; 0.20; 0.30; 0.32; 0.57; 4.0 and 4.7 μM respectively. Uptake of [^{3}H]5HT was also dependent on Cl^- as well as Na^+, and the effects of omission of both ions was non-additive. Varying media K^+ in the range of 1 to 50mM had no significant effect on [^{3}H]5HT uptake, suggesting both a lack of a specific effect of K^+ and a lack of effect of the membrane potential on 5HT uptake. These data show that uptake of serotonin in the 0.01 to 1μM concentration range by primary astrocyte cultures, based on kinetic parameters, ionic dependence and the pharmacology of inhibition, is indistinguishable from high affinity uptake reported for brain. High affinity serotonin uptake has been thought to be an exclusively neuronal property, but the present findings suggest that glia, and in particular astroglia, may play a significant role in the control of extracellular levels of serotonin in the mammalian CNS by intracellular accumulation, presumably followed by subsequent metabolism.

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221.10 Chemotaxis of Rat Brain Astrocytes to Platelet Derived Growth Factor (PDGF) and Fibronectin

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Using a purified population of rat brain astrocytes prepared from neonatal cortex, we investigated the chemotactic response of astroglia to several well characterized growth and attachment factors. Chemotactic activity for astrocytes was found for the PDGF with a half maximal response occurring at 1-2 ng/ml as compared with a value of 2-3 ng/ml obtained for Balb/c 3T3 fibroblasts in control experiments. Affinity purified fibronectin was also found to stimulate the migration of astroglia, with half maximal doses of approximately 1 ug/ml relative to maximal responses to PDGF. Fibronectin was also found to stimulate the attachment of astrocytes to types I, IV and V collagen. Several other factors including laminin, nerve growth factor, epidermal growth factor and insulin were not active as chemoattractants for astrocytes. Both nerve growth factor and laminin stimulate neurite extension, suggesting that there are specific factors controlling the movement of excitable cells during nerve regeneration. These results suggest that chemotactic factors may play a key role in the regeneration of nervous tissue.

221.11 GLIA RESPONSE TO SERIAL LESIONS IN THE DORSAL HIPPOCAMPUS OF RATS. A. Shemer*, A. Ramirez*, F.C. Zhou, and E.C. Azmitia (SPON: B. Cohen). Department of Biology, New York University, New York, NY 10003.

Simultaneous bilateral lesions in the brain can cause severe behavioral deficits. If the same lesions are carried out in serial, with 8 to 30 days between the two lesions, less behavioral deficits are produced. The physiological substrate underlying this phenomena is still unclear. We therefore decided to look into the astrocyte response as a possible factor involved in serial lesion effects.

Three groups of Sprague-Dawley rats weighing 250 gms were subjected to stereotoxic lesions to the hippocampus (coordinates at 90°: 4.5 mm anterior, 1.5 mm lateral, 4.0 mm vertical from bregma suture), while anesthetized (Chlorapent). Group 1 were lesioned unilaterally on day 1 and allowed 14 days rest. On day 15 group 1 rats received a second lesion to the contralateral hippocampus; group 2 rats received bilateral lesions; group 3 received unilateral lesions. Fourteen days after last injections, all rats were perfused intracardially with 4% paraformaldehyde in phosphate buffer for 30 minutes and brains were removed. Serial sections were cut in a coronal plane on the Oxford vibratome. Immunocytochemistry was performed using an antiserum to Glial Fibrillary Acidic protein (GFA) for 18 hrs; sheep anti rabbit for 30 minutes; and peroxidase antiperoxidase for 1 hr. Sections were washed with PBS in between reactions. The final reaction was with 3, 3' diaminobenzidine in TBS with 0.006% H_2O_2 for 5 minutes.

From preliminary studies it appears that there are fewer GFA positive cells surrounding the second serial lesion than those surrounding the first serial lesion or the unilateral and bilateral lesions.

The effects of neurotoxic (5,7-Dihydroxytryptamine) serial lesions have been investigated. We hope to compare the effects of neurotoxic lesions with mechanical lesions.

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221.12 REGULATION OF INTERMEDIATE FILAMENT PROTEIN PHOSPHORYLATION IN CULTURED ASTROGLIA. R. Pollenz* and K.D. McCarthy* (SPON: P. Trimmer). Dept. of Pharmacology, Univ. North Carolina at Chapel Hill, Chapel Hill, NC 27514.

Recent studies indicate that increasing cyclic AMP levels in astroglia result in the phosphorylation of glial fibrillary acidic protein (GFAP) and vimentin; two intermediate filament proteins present in cultured astroglia. Experiments were designed 1) to determine if the increase in phosphorylation of these proteins was due to an increase in the turnover or the number of phosphate groups and 2) to examine if the phosphorylation of these proteins was necessary for the shape change which occurs when astroglia are placed in serum-free media.

Previous experiments indicated that $^{32}P_i$ equilibrated with cellular ATP pools within 3 hr of addition to culture medium. In contrast, 6-8 hr were required to reach steady-state phosphate labelling of GFAP and vimentin. When astroglia were maintained for 16 hr in ^{32}Pi supplemented media and then stimulated for 20 min with forskolin (10 uM), the specific activity of GFAP and vimentin increased 4-fold and 2-fold, respectively. These results indicate that agents which elevate cyclic AMP levels in astroglia increase the net number of phosphate molecules on GFAP and vimentin rather than only increase the turnover of phosphate of these proteins. Forskolin exerted its maximal effect on GFAP/vimentin phosphorylation within 20 min and the effect was maintained for at least 6 hr.

Maintenance in serum-free media or stimulation of cyclic AMP levels result in the conversion of polygonal astroglia to process-bearing cells. Experiments were completed to examine the possibility that these two treatments influence cell shape via the phosphorylation of GFAP and/or vimentin. Astroglia were incubated for 16 hr in low-phosphate MEM supplemented with ^{32}Pi and 10% dialyzed FCS. Cultures were split into two groups, one group receiving the same medium and the other receiving a similar medium lacking FCS. Results indicated that switching to serum free medium led to a morphological change in astroglia prior to an increase in phosphorylation of GFAP/vimentin. Treatment of process-bearing astroglia (0% FCS) and polygonal astroglia (10% FCS) with forskolin resulted in a similar degree of stimulation of phosphorylation of GFAP and vimentin. These studies suggest that the sites on GFAP and vimentin which are phosphorylated in response to forskolin are probably not involved in the shape change which occurs after switching astroglia from 10% to 0% FCS. Supported by NS16992.

221.13 BINDING OF ^{3}H-ALDOSTERONE TO TYPE I CORTICOSTEROID RECEPTORS IN RAT C6 GLIOMA. K. Beaumont and D.D. Fanestil*. Dept. of Medicine, Univ. of Calif., San Diego, La Jolla, CA 92093.

Aldosterone (ALDO) binds to two receptor populations in rat brain cytosols. Low affinity binding is apparently to Type II corticosteroid receptors, which have higher affinity for "glucocorticoids" such as triamcinolone (TRIAM), dexamethasone (DEX), and RU 26988. A low density site (B_{max}=33 fmol/mg pro) has a high affinity (K_D=0.28nM) for ^{3}H-ALDO, like the Type I corticosteroid receptor of the rat kidney that binds "mineralocorticoids" (Beaumont & Fanestil, Endocrinol. 113:2043, 1983). Type I receptors in brain and kidney appear to share the same intrinsic specificity [deoxycorticosterone(DOC)≥corticosterone(B)≥ALDO>>DEX> TRIAM], although the steroid-sequestering action of transcortin alters the specificity of renal receptors measured in vivo or in situ to the classical "mineralocorticoid" pattern: ALDO>DOC>>B (Krozowski & Funder, PNAS 80:6056,1983).

We have measured ^{3}H-ALDO binding to C6 rat glioma cells and cytosol preparations to determine whether Type I receptors are present in these cells. Cytosols were prepared by homogenizing cells in TES/EDTA/NaMolybdate/monothioglycerol and ultracentrifugation. Cytosols were incubated for 20 hrs at 4°C with ^{3}H-ALDO in the presence of 10^{-8}M RU 26988 to block Type II receptors. Receptor-bound steroid was measured by dextran-coated charcoal. C6 glioma cytosols contained a high affinity receptor with K_D=0.26nM and B_{max}=6.3 fmol/mg pro. Rank order of steroids in competing for ^{3}H-ALDO binding was DOC>B>ALDO>DEX>TRIAM. Thus, C6 glioma and rat brain cytosolic receptors share the same high affinity for ^{3}H-ALDO and specificity for steroids. Specific (±10^{-6}ALDO) accumulation of ^{3}H-ALDO (0.05-1nM) by whole glioma cells at 37°C was rapid and blockable by 10^{-8}M spironolactone but not 10^{-8}M RU 26988.

Adrenal steroids and synthetic steroids such as DEX and TRIAM induce glycerol-3-phosphate dehydrogenase and glutamine synthetase activity in C6 glioma via interaction with "glucocorticoid" Type II receptors. The present results suggest that adrenal steroids may also affect glial cell function through an interaction with Type I corticosteroid receptors, which regulate Na^+ and K^+ transport in renal epithelial cells. C6 glioma may provide a good model system for studying the functions of Type I corticosteroid receptors located in the brain. Supported by NIH SCOR #HL 25-457.

221.14 VOLTAGE DEPENDENT Ca^{2+} CHANNELS IN GLIAL CELLS. B.A. MacVicar. Dept. of Med. Physiol., U. of Calgary, Calgary, Alta. T2N 4N1

Glial cells, the silent ubiquitous companions of neurons, are believed to be electrically inexcitable. However, the high resting gK^+ would shunt and mask any voltage dependent conductances. For this reason I have examined the properties of glial cells in culture under conditions to maximize observation of responses due to voltage dependent Ca^{2+} channels.

Glial cells, prepared from neonatal rats, were maintained in primary cultures. Glial cells were identified by their typical morphology of rounded cell body with radial processes following exposure to dibutyryl-cAMP. Some cells, which were recorded from, were immunohistochemically stained for glial fibrillary acidic protein, a specific glial marker, to ensure that recorded cells were glial cells. Cells were impaled with two microelectrodes, one for current injection and sometimes Lucifer yellow staining and the other for voltage recording.

In control solution (5 mM K^+) intracellular recordings revealed that the cells had properties identical to previous reports for glial cells (membrane potential, 77.8±4.8 mV; R input, 4.1±2.1Ω). In no cells were action potentials recorded in control solutions. To test for voltage dependent Ca^{2+} channels, recordings were obtained when TEA (5 mM) and Ba^{2+} (5-10 mM) were superfused. TEA blocks Ca^{2+} activated K^+ channels in glia (Quandt and MacVicar, this meeting). In this solution glial cells depolarized approximately 25-30 mV and R input increased 76%. Injection of depolarizing current in these previously silent cells now evoked regenerative action potentials (Ba^{2+} spikes) with durations up to several seconds. These Ba^{2+} spikes were observed in the presence of TTX (10^{-6}M) and therefore were not due to sodium channels. Spontaneous and rhythmic Ba^{++} spikes were observed in some cells. These responses were most likely due to a voltage dependent Ca^{2+} channel because they were blocked in the presence of Mn^{2+} or Cd^{++}.

These results suggest that glial cells under certain conditions could actively control extracellular K^+ or amplify K^+ signals and not just passively redistribute K^+. Activation of Ca^{2+} channels in the glial syncytium could alter the kinetics and pattern of control of extracellular ions such as K^+. Therefore glial Ca^{2+} channels may be important in the regulation of excitability of CNS structures. Abnormalities may result in hyperexcitability disorders such as seizure.

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DEVELOPMENT AND PLASTICITY: AUTONOMIC NERVOUS SYSTEM

222.1 PINEAL-SUPERIOR CERVICAL GANGLION CO-GRAFTED TO THE IV VENTRICLE, M. Brightman*, S. Markey* and D.C. Klein* (Spon: M. Whitnall). Lab. of Neurobiology, Natl. Institutes of Health, Bethesda, MD 20205.

Neural tissue, grafted to the IV ventricle is in a position to interact with the extracellular fluid contents of a relatively intact cerebral parenchyma. During fetal life, the pineal anlage of the rat is bathed by ventricular fluid. Accordingly, neonate and 4 week old pineal glands were transplanted to the IV ventricle of adult, pinealectomized rats in order to see whether the grafted cells survive and whether they can functionally replace the missing gland. Whole pineal glands were allografted to the IV ventricle, by inserting them between the vermis of the cerebellum and the medulla of adult recipients. Three weeks to three months after transplantation, the functional state of the grafts was monitored by measuring the amount of 6-hydroxymelatonin (6HO-M) in the urine over a 24 hour period. A single pineal graft produced no detectable 6H-O-M. Two pineal grafts secreted from 5 to 35ng. It was not until 6 to 8 pineal grafts had been inserted did the urinary levels reach 29 to 102ng, compared with the normal range of 120 to 300ng achieved by a single gland in situ.

Morphologically, the grafted pineals retained much of their normal organization: cords of cells, some perivascular, throughout a well vasularized, ovoid mass of tissue. The cells were identified as pinealocytes by the immunocytochemical localization of antigen "S", which is presumed to be rhodopsin kinase, and by the presence of synaptic ribbons in a few of the cells. Many of the blood vessels were fenestrated and surrounded by a generous perivascular space, as in normal glands. In hosts that had been ganglionectomized bilaterally, and in intact recipients as well, SCG fragments from one ganglion were placed against the pineal grafts. Before fixation with aldehydes, a number of these rats were given a total of 100mg of 5-hydroxydopamine intraperitoneally. In all recipients of the dual grafts, many bundles of unmyelinated axons enclosed by Schwann cells had infiltrated the pineal grafts. Some of the axons contained synaptic vesicles labeled by the amine. Neurite bundles terminated near the basal lamina of blood vessels while others lay next to a basal lamina fronting pinealocytes. The pineal transplants had become well innervated by the SCG grafts but secreted only a fraction of the melatonin produced by glands in situ.

222.2 DENDRITIC DEVELOPMENT IN THE RAT SUPERIOR CERVICAL GANGLION. A. J. Smolen and P. Beaston-Wimmer*. Dept. of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Previous studies from our laboratory have shown that synaptogenesis in the superior cervical sympathetic ganglion (SCG) of the rat occurs predominantly during the first few weeks after birth. The purpose of the present study was to examine the normal development of dendrites of the ganglion neurons, and to assess the importance of the afferent input in shaping this development.

We have employed two independent methods for examining dendritic morphology. One is to label neurons in the SCG by injecting a conjugate of horseradish peroxidase and wheat germ agglutinin (HRP-WGA) into a target of the SCG neurons (the submandibular gland or the iris). This procedure results in a "Golgi-like" filling of the retrogradely labelled cell bodies and their dendrites. The second method is the use of stereologic analysis (point-counting) of electron micrographs of sections of the SCG.

At birth, HRP-WGA retrogradely labels many neurons in the SCG which project to the submandibular gland and the iris. The cell bodies of the ganglion neurons are small (about 12-14 um in diameter), and give rise to several short (under 15 um), thin dendrites. Electron microscopic stereology reveals that the mean volume of neuron cell bodies in the SCG is about 2300 um^3. The mean volume of dendrites in the newborn is about 200 um^3 per neuron.

In the adult, HRP-WGA labelled neurons have significantly larger cell bodies (about 25-30 um in diameter), and several stout primary dendrites which can be followed for 30-50 um before arborizing into smaller processes. Stereological analysis shows that, between birth and adulthood, there is a four-fold increase in the mean volume of cell bodies (to 9000 um^3 per neuron) and a much larger (12-fold) increase in the mean volume of dendrites (to 2800 um^3 per neuron).

In ganglia from adult rats which were deafferented at birth, stereologic analysis demonstrated that normal dendritic maturation occurred. Retrograde labelling studies are currently in progress with these deafferented ganglia to determine whether there is any alteration in the dendritic configuration.

We conclude that, while dendritic development in the SCG is mainly a postnatal event which occurs at the same time as synaptogenesis, this development appears to be independent of presynaptic influences.

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222.3 CRITICAL PERIOD IN THE DEVELOPMENT OF ACH SENSITIVITY BY MAMMALIAN SENSORY NEURONS IN CULTURE. E. Cooper and M. Lau*. Department of Physiol. McGill University, Montreal, Quebec, Canada H3G 1Y6.

The nodose ganglia is an autonomic sensory ganglion whose axons innervate the viscera. Like other peripheral sensory ganglia in mammals, the neurons in this ganglia do not receive synapses. Interestingly, nodose neurons from newborn rats form synapses with one another when the neurons develop in tissue culture; these synapses are reversibly blocked by nicotinic antagonists, indicating that some neurons release ACh and that the postsynaptic receptors are nicotinic. As synapses do not form on these neurons in vivo, most, if not all, the postsynaptic elements at these synapses develop in culture. Functional synapses, however, do not form among these sensory neurons when they are co-cultured with the ganglionic satellite cells, in part, because most neurons are not sensitive to ACh: only 5-20% of neurons co-cultured with satellite cells are ACh-sensitive, whereas approximately 75-85% of neurons cultured without nonneuronal cells are ACh-sensitive.

As the ACh receptors play an important role in the formation of these synapses, experiments are in progress to investigate the development of ACh-sensitivity by these sensory neurons. At each plating all cultures contain both neurons and ganglionic satellite cells initially; 2 days later the cultures are divided into 2 groups: one group is treated to kill off the satellite cells, leaving the neurons to develop by themselves; in the other group, the neurons and the ganglionic satellite cells develop together. 8-10 days after plating only a small percentage of neurons, approx. 10-20%, are ACh-sensitive in both types of cultures. One week later, however, over 80% of the neurons in cultures without satellites cells are ACh sensitive, whereas the % ACh-sensitive in cultures with satellite cells remain unchanged at 10-20% for at least another 3 weeks. In other experiments, the neurons were first co-cultured with satellite cells for 10 days, thereafter, the neurons developed by themselves for a further 3 weeks; most neurons in these cultures remained insensitive to ACh. In other cultures, the neurons initially developed without other cell types and then the satellite cells were added 8-10 days later, and the neurons together with the satellite cells were co-cultured for another 3 wks; most neurons (75-85%) in these cultures were sensitive to ACh. Supported by MRC of Canada.

222.4 VAGUS NERVE STIMULATION MODIFIES THE ELECTRICAL ACTIVITY OF THE OLFACTORY BULB. D. E. García-Diaz*, H. U. Aguilar-Baturoni, Rosalinda Guevara-Aguilar and Matthew J. Wayner. Depto. de Fisiología, Facultad de Medicina, UNAM 04510, México, D. F. Division of Life Sciences University of Texas at San Antonio San Antonio, Texas 78285 U.S.A.

The evoked potential and the unit activity recording techniques were used to study the vagus nerve influences upon the olfactory bulb. A biphasic potential was evoked in the olfactory bulb by a single pulse delivered to the vagus nerve. Half of the recorded neurons decreased their discharge frequency after single pulse or train stimulation. The lapse in which the firing rate of the neurons was stopped paralelled the duration of the negative wave of the evoked potential. The responsive neurons were iontophoreticaly labeled with horseradish peroxidase and were located in the periglomerular layer of the olfactory bulb. These results suggest the existence of a vagus nerve-olfactory bulb pathway.

222.5 ß-ADRENERGIC RECEPTOR DENSITY IN SYMPATHETICALLY-DENERVATED AND SYMPATHETICALLY-ANEURAL EMBRYONIC CHICK HEARTS. D. E. Stewart and M. L. Kirby. Department of Anatomy, Medical College of Georgia, Augusta GA 30912.

ß-Adrenergic receptor density in certain tissues of adult animals can be modified by peripheral administration of catecholamines and surgical or chemical sympathetic denervation of the tissue being studied. The aim of this investigation was to determine whether ß-adrenergic receptor density in embryonic chick heart is modified by surgical or chemical sympathectomy in the same manner. Sympathetically-aneural hearts were produced by ablation of neural crest cells over somites 10-20 and somites 5-25. Ablation of neural crest over somites 10-20 removes sympathetic innervation of the developing chick heart without modifying cardiac norepinephrine concentration. Lesion of neural crest over somites 5-25 decreases cardiac norepinephrine in addition to removing sympathetic cardiac innervation. Chick hearts were collected for ß-adrenergic receptor-binding assays on incubation day (ID) 16 or 17. Specific ß-adrenergic receptor binding was defined as total (^{125}I) l-pindolol binding minus binding in the presence of 50 µM isoproterenol. No difference in ß-adrenergic receptor concentration in whole hearts was observed between animals lesioned over somites 5-25 (42.0 ± 6.1 fmol/mg protein) and unoperated control animals (49.3 ± 6.8 fmol/mg protein). Likewise, animals lesioned over somites 10-20 had the same density of cardiac ß-adrenergic receptors as unoperated controls (10.4 ± 1.2 pmol/mg protein versus 11.4 ± 1.2 pmol/mg protein, respectively). Sympathetically-denervated hearts were produced by administration of 6-hydroxydopamine (6-OHDA, 100 mg/kg daily) to embryos on ID 13-19. Hearts were collected on ID 20 for receptor assays. Saline-treated embryos had the same cardiac ß-adrenergic receptor concentration as 6-OHDA-lesioned embryos (30.5 ± 6.5 fmol/ mg protein versus 28.9 ± 8.7 fmol/mg protein, respectively). In contrast to adult animals, this data indicates that ß-adrenergic receptor density in embryonic tissue is not modified by surgical or chemical sympathectomy, nor is it responsive to changes in cardiac norepinephrine concentration.
Supported by NIH grant HD 17063.

222.6 ABLATION OF NEURAL CREST TO ELIMINATE SYMPATHETIC INNERVATION OF THE DEVELOPING CHICK HEART. M. L. Kirby and D. E. Stewart. Department of Anatomy, Medical College of Georgia, Augusta GA 30912.

The major drawback in studying autonomic interactions during development has been the inability to remove one or both divisions of the autonomic system which innervate a particular organ. We have recently produced parasympathetically aneural hearts by removal of a specific region of premigratory occipital neural crest. The aim of the present investigation was to locate and remove the neural crest responsible for sympathetic innervation to the heart in order to produce sympathetically aneural hearts. The sympathetic cardiac innervation arises bilaterally from the first thoracic sympathetic ganglia. Neuronal uptake of (^{3}H)-norephinephrine was used as an index of neuronal development in the chick atrium. Uptake was significantly decreased in the atrium at 16 and 17 days of development following ablation of neural crest over somites 10-15 or 15-20. Ablation of neural crest over somites 5-10 or 20-25 caused no change in atrial norepinephrine uptake. Removal of neural crest over somites 5-25 or 10-20 caused approximately equal depletions of uptake. Cardiac norepinephrine concentration was not significantly decreased following ablation of neural crest over somites 10-20 but was significantly decreased by lesion of neural crest over somites 5-25. Light and histofluorescence microscopy confirmed the absence of sympathetic trunks in the thoracic region and innervation to the atria. This indicates that the cardiac innervation orginates from cells in the sympathetic trunks which arise from neural crest over somites 10-20. A lesion of neural crest over somites 5-25 would eliminate the adrenal medulla as well as the cardiac sympathetic innervation. Residual norepinephrine in the heart following lesion of the sympathetic cardiac innervation probably derives mainly from the adrenal medulla.
Supported by NIH grant HD 17063.

222.7 DEVELOPMENT OF ENTERIC GLIA IN NORMAL MURINE BOWEL AND IN THE CONGENITALLY AGANGLIONIC COLON OF LETHAL SPOTTED (ls/ls) MUTANT MICE. T.P. Rothman, G. Nilaver, C.J. Haaksma and M.D. Gershon. Depts. of Anatomy and Cell Biology, and Neurology, Columbia University, College of Physicians & Surgeons, New York, NY 10032.

The enteric nervous system (ENS) is a unique portion of the PNS that differs from other regions physiologically, chemically, and structurally. Among these differences are the presence within the ENS of enteric glia. These cells resemble CNS astrocytes and differ from Schwann cells in having no basal laminae and dense bundles of intermediate filaments that contain large quantities of glial fibrillary acidic protein (GFAP). The cells can therefore be distinguished from Schwann cells by their far greater GFAP immunocytochemical reactivity. We investigated the development of enteric glia in normal and ls/ls mice. The ls/ls animals develop aganglionic segments of terminal bowel. These segments receive axons projecting to the gut from extrinsic ganglia but contain no intrinsic enteric neurons. We have found that the precursors of enteric neurons fail to enter the presumptive aganglionic ls/ls gut. During development GFAP immunoreactivity appears as a late marker and is not seen until day E19, long after neuronal phenotypic expression has become evident. Nevertheless, GFAP immunoreactivity appeared within 12-14 days in cultured explants of gut removed before GFAP expression could be detected in situ; therefore, the precursors of enteric glia colonize the gut before they express GFAP. In fact, glial precursors could be detected by this explant assay in the entire gut of normal mice, as early as day E10-E11. This timing indicates that glial as well as neuronal precursors are present in the bowel before either end stage cell is apparent. In contrast to the ganglionated proximal bowel, which always provided cultures with glia, the terminal, presumptive aganglionic ls/ls gut generally yielded cultures devoid of glia (25/27) as well as neurons (27/27). Despite these results, the supporting cells of extrinsic nerves in adult ls/ls terminal bowel displayed the GFAP staining pattern of enteric glia, not Schwann cells. These experiments indicate that glial precursors can enter and survive in the aganglionic bowel of ls/ls mice even though neural precursors cannot. These cells of adult colon may be derived from Schwann cells entering the gut with the extrinsic innervation. Supported by NSF grant BNS 83-04904, NIH grant NS 15547, MOD grant 1-747, Dysautonomia Fdn.

222.8 STRUCTURE OF THE SMALL, GRANULE-CONTAINING CELLS IN THE SUPERIOR CERVICAL GANGLIA OF HYDROCORTISONE-TREATED EARLY POSTNATAL AND ADULT RATS. H. Päivärinta[x], S. Soinila[x] and O. Eränkö[x]. (SPON: P. Panula). Department of Anatomy, University of Helsinki. Siltavuorenpenger 20 A, 00170 Helsinki, Finland.

Hydrocortisone causes an increase in the number of small intensely fluorescent (SIF) cells and the appearance of phenylethanolamine-N-methyltransferase (PNMT) immunoreactivity in them in the rat superior cervical ganglion. This sensitivity to glucocorticoids has been reported to be lost soon after birth, but retained if the rats have been "primed" neonatally with glucocorticoids.

In the present study the effect of hydrocortisone has been studied on the fine structure of the small, granule-containing (SGC) cells, corresponding with the SIF cells ultrastructurally, in the rat superior cervical ganglion early postnatally and in the adult rat.

Hydrocortisone injections into rats on postnatal days 3-9 caused an increase in the number of the SGC cells as observed on the 10th day. These cells showed an extensive rough endoplasmic reticulum, a large Golgi apparatus and a very large number of granular vesicles. In addition to the granular vesicles, 70-160 nm in diameter, in which the dense core filled most of the vesicle, most cells of the hydrocortisone-injected rats contained also larger granular vesicles, up to 350 nm in diameter, in which the dense core was eccentrically located. A minority of cells contained only granular vesicles 70-100 nm in diameter. The latter was the only type of granule in the SGC cells seen in ganglia of 10-day-old saline-treated control rats. Thirty days after discontinuation of the hydrocortisone treatment, most of the cells with large granular vesicles had dissapeared. Hydrocortisone treatment, first on days 3-9 and secondly on days 40-46, caused reappearance of SGC cells with large granular vesicles. Hydrocortisone treatment on days 40-46 alone did not cause appearance of such cells in rats treated with saline on days 3-9.

It is concluded that the responsiveness to hydrocortisone-induced increase in the number of SGC cells with large granular vesicles is lost during normal development, but the response to hydrocortisone can be obtained in young adult rats if these have been subjected to early postnatal hydrocortisone treatment.

222.9 INNERVATION OF BRACHIAL SYMPATHETIC GANGLION CELLS IN NORMAL AND WING-EXTIRPATED CHICKS. J.W. Yip and Katherine Klein*, Dept. of Physiol. Sch. of Med., Univ. of Pittsburgh, Pittsburgh, PA 15261.

Wing-bud removal in the chick embryo results in a 57.6% reduction (N=4) in the number of neurons in the fifteenth cervical sympathetic ganglion (C15) of the post-hatched chick. We report here the innervation of the remaining ganglion cells by preganglionic axons.

The right wing-buds of stage 17-19 (2 1/2-3 days) chick embryos were removed. The operated embryos were returned to the incubator and allowed to hatch. One to three months after hatching, the fifteenth cervical sympathetic ganglion of both control and experimental animals were dissected in continuity with the thoracic portion of the sympathetic chain. Intracellular recordings were made from individual ganglion cells while stimulating the ventral roots of the spinal segments which contribute innervation to the ganglion.

As in mammals, each sympathetic ganglion cell of the normal chick is innervated by a subset of the spinal segments that supply the ganglion as a whole. The subset of spinal segments is contiguous, with one segment providing the dominant innervation to the cell. Impalement of 205 cells in normal C15 ganglia indicated that each ganglion cell is contacted on average by 11.6 ± 0.2 axons arising from 3.5 ± 0.06 contiguous spinal segments derived from C15-T4. The pattern of ganglion cell innervation remained largely the same in wing-extirpated animals. 226 cells were impaled in ganglion C15 of wing-extirpated animals. Despite a reduction in the number of ganglion cells, each cell is contacted on average by 12.8 ± 0.25 axons arising from 3.27 ± 0.06 spinal segments. The average size of the EPSP recorded in individual neurons of normal and wing-extirpated animals also remains unchanged; their respective values being 29.1 ± 0.67 and 29.9 ± 0.75mV.

These results suggest that in the sympathetic system of the chick, preganglionic neurons respond to a reduction of the postganglionic population by reducing either the number of neurons or the number of synapses each neuron makes. (Supported by BNS-8210028).

222.10 RECOVERY OF CARDIOVASCULAR FUNCTION AFTER PARTIAL SYMPATHECTOMY: POSSIBLE ROLE OF INCREASED TYROSINE HYDROXYLASE. S.J. Fluharty,* R.R. Vollmer,* M.J. Zigmond & E.M. Stricker. (SPON: R.G. MacKenzie). Depts. of Biological Sciences & Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Catecholamine (CA)-containing neuronal systems can sustain substantial damage without severe disturbance of function. We have proposed that such plasticity results from biochemical adaptations to the injury that occur within residual elements of the damaged system. We are evaluating this hypothesis by examining cardiovascular function in rats after systemic 6-hydroxydopamine (6-HDA) treatment. Male rats (250-300 g) received 6-HDA (100 mg/kg, sc), a treatment which initially reduced the CA content of the heart by 94% without affecting adrenal CA levels. Three days after 6-HDA treatment we recorded heart rate and blood pressure responses to a range of stimulation frequencies (0.25-8.0 Hz), delivered by way of a pithing rod inserted in the thoracolumbar region of the spinal cord. We observed a marked decrease in the pressor responses of 6-HDA-treated rats. In contrast, the heart rate responses were much less affected. While at low frequencies of stimulation, the tachycardia responses ranged from 20-40% of control at higher frequencies the increases in heart rate were normal. The maintenance of chronotropic responsiveness may have been mediated by enhanced adrenal medullary CA secretion because adrenal TH activity was increased by 68% 3 days after 6-HDA treatment and plasma epinephrine levels during stimulation were increased markedly.

Three weeks after 6-HDA treatment, both pressor and heart rate responses to stimulation were normal. Adrenal medullary influences appeared to be less important then. Instead, restoration of function may have resulted from enhanced synthesis and release of CA from surviving sympathetic terminals. Indeed, although cardiac CA content was decreased by 83% at this time, TH activity was reduced by only 35%, suggesting that TH activity was increased substantially within undamaged neurons of the heart. This local enhancement of CA synthesis developed more gradually than that which occurred in the adrenal medulla but it persisted for several weeks longer. These results further attest to the impressive functional plasticity of partially damaged CA systems. In addition, they provide evidence that biochemical adaptations that permit enhanced synthesis and secretion of CA within the sympathetic nervous system may have a functional impact on denervated target organs.

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222.11 RESPONSES OF CULTURED RAT SUPERIOR CERVICAL GANGLIA TO NERVE GROWTH FACTOR, HYDROCORTISONE AND HEART ATRIUM EXPLANTS. S. Soinila°, H. Päivärinta° T. Lahtinen° and O. Eränkö° (SPON:B.Wise) Department of Anatomy, University of Helsinki, Siltavuorenpenger 20 A, SF-00170 Helsinki, Finland.

Evidence has been presented to suggest that the nerve growth factor (NGF) and glucocorticoids exert competitive influences on the sympathetic precursor cells resulting in induction of neuronal phenotype in the principal nerve (PN) cells and neuroendocrine phenotype in the small intensely fluorescent (SIF) cells. The target tissues of the sympathetic ganglia produce NGF and possibly also other growth factors which are thought to attract fibre growth from the PN cells.

This study reports on the effects of exogenous NGF and hydrocortisone (HC) or heart atrium explants on cultured pre- and postnatal superior cervical ganglia of the rat.

NGF produced extensive nerve fibre growth from the PN cells first in ganglia of 15-day-old (E15) embryos, while culture with HC resulted in increased SIF cell numbers even in E13 ganglia. HC no longer induced increased SIF cell numbers in the ganglia of 8-day-old and older postnatal rats. Neither NGF nor its antiserum affected survival of the SIF cells. HC did not essentially affect fibre growth from the PN cells. Culture with both NGF and HC showed that neither NGF nor its antiserum prevented HC from inducing new SIF cells, and NGF failed to enhance fibre growth from HC-induced SIF cells. HC did not affect NGF-induced fibre growth from the PN cells.

Newborn atrium explants elicited fibre growth from both newborn and E15 ganglia, and this effect was totally abolished by anti-NGF. In contrast, fibre growth from E15 ganglia induced by E15 atria was only partially inhibited by anti-NGF.

The present results suggest that (1) NGF and glucocorticoids may affect different cell populations rather than competitively influence the same precursor cells, and (2) growth factors other than NGF may take part in the regulation of nerve fibre growth from early prenatal sympathetic ganglia.

DEVELOPMENT AND PLASTICITY: TRANSMITTER PHENOTYPIC PLASTICITY II

223.1 PARTIAL EXPRESSION OF CATECHOLAMINERGIC TRAITS IN CHICK EMBRYO CILIARY GANGLIA. G. Teitelman, V. Albert, T.H. Joh, D.J. Reis and L. Iacovitti. Cornell Univ. Med. Coll., New York, NY 10021.

We have previously reported that cells of the cholinergic ciliary ganglia (CG) of chick embryo contain tyrosine hydroxylase (TH), but not phenylethanolamine N-methyltransferase (PNMT) during development in vivo and that in vitro CGs from E8 chicks express both catechoamine (CA) enzymes (Iacovitti et al., Neuroscience Abstr. 304, 1983). In this study, we examined, first, whether cells of the CG contain aromatic L-amino acid decarboxylase (AADC), another CA enzyme. To do so, CGs were removed from E5 to postnatal day 10 (P10), fixed and processed for the immunocytochemical localization of AADC. At all stages examined, CG neurons stained with AADC although the staining was less intense in ganglia from postnatal than from embryonic chicks. In CGs removed from E8 embryos and maintained for 5 days in vitro, all neurons contained AADC while non-neuronal cells were devoid of stain. The fact that cultures of CG incubated with the amine precursor L-DOPA exhibit dopamine histofluorescence indicates that AADC is active. However, CG neurons in vitro do not contain endogenous CAs, nor do they take up and store exogenous CAs. Second, we examined whether CG neurons in vitro can simultaneously express catalytically active adrenergic and cholinergic enzymes. To do so, the activity of TH and of choline acetyltransferase (CAT) was measured in cultures of E8 CGs. After 5 days in vitro, CAT activity was (51.4 + 3.1, fmols/neuron/hr) and while TH activity was undetectable, all neurons of corresponding sister cultures contained TH immunoreactivity. Third, we sought to determine which cell type of the CG in vivo initiates the expression of TH once the ganglia is placed in culture. Ganglia removed from E8 chicks and maintained throughout the culture period in media containing ^{3}H or ^{14}C thymidine were processed for immunostaining and radioautography. In all cases cells containing TH immunoreactivity were devoid of silver grains. This finding indicates that the CA traits are expressed by the postmitotic neurons of the CG.

We conclude that the postmitotic neurons of the CG are able to express some but not all of the traits characteristic of a CA phenotype while maintaining cholinergic expression. These findings suggest that 1) the appearance of the full complement of adrenergic properties is not coordinated and may be regulated by different environmental cues and, 2) CG neurons can express both adrenergic and cholinergic traits simultaneously. (Supported NIH Grant HL18974 and NSF Grant PCM8303019.)

223.2 DEVELOPMENT OF CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE SYMPATHETIC INNERVATION OF RAT SWEAT GLANDS. G.G.Leblanc* and S.C.Landis (SPON:M.S.Livingstone). Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Previous morphological studies have provided evidence that the sympathetic neurons which innervate rat sweat glands (SGs) switch their neurotransmitter phenotype from adrenergic to cholinergic during postnatal development (Landis and Keefe, Dev. Biol. 98, 349). In order to define the time of onset of cholinergic properties in the SG innervation, we have examined the development of choline acetyltransferase (CAT) activity in these neurons.

CAT activity was assayed in homogenates of gland-rich chunks of footpad tissue according to the method of Fonnum (J. Neurochem. 24, 407). Measurements were begun at day 4, the time at which sympathetic axons first arrive at the developing SGs. Apparent CAT activity was extremely low until day 14, and began to rise between days 14 and 21. CAT activity continued to increase in a linear fashion for up to 42 days, the latest timepoint studied. Total gland protein also increased linearly during this period, but at a slower rate than did CAT activity. Thus, there was a 4-fold increase in specific CAT activity between days 21 and 42.

To determine whether the low level of acetylating activity observed prior to day 21 is due to the presence of CAT in the sympathetic innervation of the SGs, neonatal animals were treated with 6-hydroxydopamine (6-OHDA)(100 mg/kg, days 1-7 and 12). This treatment abolishes both the developing and mature sympathetic innervation of the SGs (Yodlowski et al., J. Neurosci., in press). The loss of innervation due to neonatal 6-OHDA treatment did not significantly affect the acetylating activity of SGs taken from 4 and 7 day animals, and only slightly decreased the activity of 14 day SGs. By 19 days, however, acetylating activity was reduced 75% in SGs from 6-OHDA-treated animals. Similarly, at 19 days, incubation of SG homogenates from control animals with the CAT inhibitor napthylvinylpyridine (PVN) resulted in a 70% decrease in acetylating activity, while PVN had little effect on the activity of SG homogenates from 6-OHDA-treated animals. Hence, it is only in the 19 day animals that significant levels of specific CAT activity can be attributed to the presence of sympathetic nerve terminals in the SGs.

The relatively late acquisition of CAT activity in the sympathetic innervation of the rat SGs provides further support for the hypothesis that these neurons undergo a transition from adrenergic to cholinergic phenotype, and suggests a role for target tissue in mediating this transition.

223.3 THE DEVELOPMENT OF CHOLINERGIC TRANSMISSION IN THE RAT ECCRINE SWEAT GLANDS L.M.Stevens*and S.C.Landis (SPON:D.D. Potter). Dept. of Neurobiology, Harvard Med. Sch., Boston, MA 02115

Previous studies have shown that the cholinergic sympathetic fibers which innervate rat eccrine sweat glands (SGs) initially express noradrenergic properties which are lost as the glands and their innervation mature. To document further the change in transmitter phenotype, we studied the onset of cholinergic transmission in developing SGs.

Rats were assayed at 16, 18, 21 and 25 days of age for the ability of both nerve stimulation and cholinergic agonists to produce a secretory response in the SGs of the plantar footpads. The sciatic nerve was stimulated for 2-5 min. at 7.5-12.5V at 5½Hz using platinum electrodes embedded in a silastic cuff. Active SGs were detected with a silicone impression material (Kennedy & Sakuta, Neurosci. Abs., 8:27, 1982). Drugs were dissolved in saline and administered locally by injecting individual footpads.

Both nerve stimulation and cholinergic agonists elicited a secretory response in 16d. old rats. Four out of 10 rats tested exhibited sweating during nerve stimulation, but active glands were present in only 1-4 of the 11 footpads. The same glands were activated by $2X10^{-5}$M muscarine. By 18d., 9 out of 10 rats tested secreted in response to nerve stimulation and injection of $1-5X10^{-5}$M methacholine. In most rats, all footpads contained some active glands. At 21 and 25d., all rats tested exhibited sweating during nerve stimulation. The number of active glands per footpad increased with age, as did sensitivity to cholinergic agonists. By 25d., some glands responded to $5X10^{-7}$M methacholine.

At all ages tested, (18, 21 and 25d.), sweating evoked by nerve stimulation was blocked by 10^{-6}M atropine, a cholinergic antagonist. In contrast, nerve-evoked secretion was unaffected by the adrenergic antagonists phentolamine and propranolol, and sweating could not be elicited by the adrenergic agonists clonidine and isoproterenol.

These results indicate that cholinergic function is present in the SGs as early as 18d. in most rats and perhaps at 16d. in some. Of 4 rats examined at 14 days, neither nerve stimulation nor $2X10^{-5}$M muscarine produced secretion. These observations are consistent with biochemical studies (Leblanc & Landis, this volume) indicating that there is little or no choline acetyltransferase activity in the SGs at 14d. and that its activity begins to increase between 14 and 19d. The time course of the onset of cholinergic transmission in the SGs supports the proposed transition in transmitter phenotype.

223.4 PLASTICITY OF PEPTIDE CONTENT IN PC-12 CELLS - ROLE OF NERVE GROWTH FACTOR.
R. Baumann-Drake*, F. Businger*, U. Otten.(SPON:W.F.Fischli) Dept. of Pharmacology, Biocenter of the University, Basel, Switzerland.

The rat pheochromocytoma clonal cell line (PC-12) has been shown to be a useful experimental system for the study of mechanisms involved in differentiation of cells derived from the neural crest. In addition to classical neurotransmitters various neuropeptides have been localized in cells of neural crest origin.
Using sensitive radioimmunoassays with sequence specific antibodies we have detected substance P-(SP), somatostatin-(SOM) and vasoactive intestinal polypeptide-(VIP) like immunoreactive material in PC-12 cells (0.124, 0.054 and 0.17 pmol/mg protein respectively).
A characteristic property of PC-12 cells is its responsiveness to growth factors and hormones. In the present study we investigated the effects of 2.5S nerve growth factor(NGF) on neuropeptide content and transmitter synthesizing enzymes such as choline acetyltransferase and tyrosine hydroxylase.
Exposure of PC-12 cells to NGF (50 - 200 ng/ml) caused a dose-dependent increase of VIP content. Within 5 days 50 ng/ml NGF caused a three-fold, 200 ng/ml NGF a six-fold increase of VIP. The increase in VIP was accompanied by a dose dependent rise in choline acetyltransferase activity. On the other hand, NGF did not affect either SOM or SP content nor tyrosine hydroxylase activity.
Our results indicate that NGF regulates VIP expression in in PC-12 cells. It remains to be determined whether other growth factors or hormones which are important determinants of transmitter phenotypes can affect neuropeptide levels in these cells.

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223.5 DEPOLARIZATION INCREASES GABA SYNTHESIS IN RETINAL CELLS IN CULTURE. B-A. Battelle. National Eye Institute, NIH, Bethesda, MD. 20205

Mechanisms which modulate the development and function of neurons in the central nervous system are largely unknown. We are studying biochemical parameters associated with the function of synapses of neurons from rat retina maintained in monolayer culture. We reported that the rate of GABA synthesis and storage in cultures of cells dissociated from embryonic rat retinas and grown in medium containing an elevated concentration of K^+ (50 mM) was twice that measured in sister cultures grown in normal medium containing 4 mM K^+. (Soc. Neurosci. Abstr. 9:1099, 1983). To further characterize this effect the following have been investigated: 1. the relationship between the rate of GABA synthesis and the concentration of K^+ in the growth medium 2. the effect of veratridine and tetrodotoxin (TTX) on the rate of GABA synthesis and 3. the effect of Ca^{++} channel blockers on K^+-stimulated increases in the rate of GABA synthesis.

Increasing the concentration of K^+ in the growth medium (HEPES-buffered F12/DMEM plus 5% fetal calf serum) from 4 to 10 mM did not significantly increase the rate of GABA synthesis. The rate of GABA synthesis increased linearly between 10 and 30 mM K^+ and was maximum between 30 and 50 mM K^+. Exposing cultured retinal cells to veratridine also increased the rate of GABA synthesis. The maximum increase obtained with 10 μM veratridine (Higher concentrations of veratridine were toxic.) was 50-60% of that obtained with 50 mM K^+. Addition of TTX to the growth medium completely inhibited the veratridine-stimulated increase in GABA synthesis and partially inhibited the K^+-stimulated increase. Addition of the Ca^{++} channel blockers D-600 ($5x10^{-6}$M) or nitrendipine ($5x10^{-9}$M) to the medium also significantly inhibited the K^+-stimulated increase in the rate of GABA synthesis.

Taken together these results suggest that depolarization increases the rate of GABA synthesis in retinal neurons and that this increase depends in part on the influx of extracellular Ca^{++}.

223.6 PHARMACOLOGICAL STUDIES OF HYPERPOLARIZING AND AGE-DEPENDENT DEPOLARIZING RESPONSES OF CULTURED LOCUS COERULEUS (LC) NEURONS TO NORADRENALINE (NA). P.G. Finlayson* and K.C. Marshall, Dept. of Physiol., Univ. of Ottawa, Ottawa, Canada K1H 8M5.

We have previously demonstrated an age-dependent change in the responses of cultured LC neurons to iontophoretically applied NA (Dev. Brain Res., in press). LC neurons in explant cultures from newborn mice were recorded intracellularly. Biphasic responses to NA, a hyperpolarization followed by a depolarization, were observed in most LC neurons grown in culture for less than 26 days, while only hyperpolarizing responses to NA were observed in LC neurons cultured for more than 26 days. We now report pharmacological evidence supporting our earlier hypothesis that the hyperpolarizing and depolarizing components of biphasic responses are mediated by different adrenoceptors.

The hyperpolarizations of cultured LC neurons by NA appear to be mediated by α_2 adrenoceptors. Iontophoretic application of clonidine, an α_2 agonist, evokes a long hyperpolarization in the LC neurons at all ages tested. Furthermore, Yohimbine (100nM-1uM), a selective α_2 antagonist, abolishes hyperpolarizations to NA and antagonizes the hyperpolarizing component of the biphasic responses without reducing the depolarizing component. Thus, as in vivo and in tissue slices, α_2 adrenoceptors mediate hyperpolarizations in cultured LC neurons.

The depolarizing responses of LC neurons to NA appear to be mediated by α_1 receptors. The β-adrenoceptor blockers, propranolol (1uM) and sotalol (1uM) had no effect on the depolarizing or the hyperpolarizing responses to NA. However, Corynanthine (10uM), an α_1 blocker and Prazocin (200 nM-1uM), an α_1 blocker, reduced or abolished the depolarizing component of biphasic responses, leaving a hyperpolarizing component. Furthermore, iontophoresis of the α_1 agonist, phenylephrine evoked small depolarizations. As the depolarizing responses of LC neurons to NA is age-dependent and there are relatively few α_1 adrenoceptors in the LC of adult animals, it is likely that a population of α_1 adrenoceptors on LC neurons are lost during maturation. These observations may indicate that in infants, noradrenergic neurotransmission in the LC has both excitatory and inhibitory effects, while after maturation NA has only an inhibitory action, as has been observed in studies of the LC from adult animals.
(Supported by the Medical Research Council of Canada; PGF is supported by an Ontario Graduate Scholarship).

223.7 TYROSINE HYDROXYLASE AND CHOLINEACETYLTRANSFERASE IN CULTURES OF RAT ENTERIC NEURONS: IMMUNOREACTIVITY AND ENZYME ACTIVITY. R. NISHI & A.L. WILLARD. Dept. of Neurobiology, Harvard Med. School, 25 Shattuck St., Boston, MA 02115

We have developed culture protocols that promote and maintain differentiation of neurons dissociated from myenteric plexus of neonatal rats. These cultured neurons have many of the differentiated properties of their in vivo counterparts, including synaptic interactions, chemosensitivity, ultrastructure and immunoreactivity to antisera against peptide transmitter candidates. We describe here results obtained with 2 other probes we have used to examine cultured enteric neurons: a rabbit antiserum to rat tyrosine hydroxylase (TH) and a mouse antiserum to pig cholineacetyltransferase (ChAT). These antisera have been characterized carefully in other systems as specific for their respective antigens (Thibeault et al, BBRC 99: 960; Eckenstein & Thoenen, EMBO J 1: 363).

As expected, the anti-ChAT labeled a major (30-60%) subpopulation of neurons in our cultures. Cells were stained by a very sensitive method using 2 cycles of PAP preceded by incubation in goat anti-mouse. The presence of ChAT-containing neurons agrees with previously obtained results from electrophysiological experiments showing that many of the neurons interacted via excitatory nicotinic synaptic connections and from biochemical experiments showing that cultures could synthesize and store ACh from exogenously supplied ^{3}H-choline. We have shown recently that electrophysiologically identified cholinergic neurons are labeled by this antiserum.

More surprisingly, we find that 6-10% of the neurons in our cultures are stained by the anti-TH. These neurons differ morphologically from those stained by anti-serotonin, suggesting that the staining is not due to cross reactivity to tryptophan hydroxylase. Cultures were shown to have TH enzyme activity by incubating them with ^{3}H-tyrosine and examining the products by high voltage paper electrophoresis. Tyrosine was converted to DOPA, but no synthesis of dopamine or norepinephrine was detected. In accord with these biochemical data, we have been unable to detect catecholamine flourescence in these cultures. Currently, we are staining whole mounts of smooth muscle strips containing myenteric plexus in order to test whether our culture conditions are inducing TH in some of the enteric neurons or whether the rat small intestine normally contains a population of enteric neurons that contain TH.

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AGING IV

224.1 NORADRENERGIC NEURON TRANSPLANTS INTO THE III VENTRICLE OF AGED F344 RATS IMPROVE INHIBITORY AVOIDANCE MEMORY PERFORMANCE. J.R. Sladek, Jr., D.M. Gash and T.J. Collier. Department of Anatomy, Univ. of Rochester Sch. of Med., Rochester, N.Y. 14642.

Transmitter content of brain norepinephrine (NE) systems declines with old age in F344 rats (Sladek and Blanchard, 1981, in: "Brain Neurotransmitters and Receptors in Aging and Age-Related Disorders", S.J. Enna et al., eds., Raven Press, N.Y., p. 13). NE function has been linked to memory for stressful avoidance situations (Gold and Van Buskirk, 1978, Behav. Biol., 23:509), and it is well-documented that aged rats exhibit marked memory deficits for inhibitory avoidance tasks (for review see Kubanis and Zornetzer, 1981, Behav. Neural Biol., 31:115). To determine whether supplementation of the aged brain's NE system could help normalize memory performance, we transplanted 15-16 day gestation fetal pontine tissue, including the region of the developing noradrenergic nucleus locus coeruleus bilaterally, into the III ventricle of six 23 month old (m.o.) F344 rats. Six weeks later subjects were trained and tested for 24 hour retention of a step-through inhibitory avoidance task. Memory performances of 5 m.o. unoperated controls (n = 10), 24 m.o. unoperated controls (n = 12), 24 m.o. recipients of NE-containing grafts (n = 6) and 24 m.o. recipients of cerebellum grafts (n = 5) were compared. Aged host and control animals were selected from a subpopulation of aged rats exhibiting gustatory neophobia: A correlate of deficient NE system function and predictor of poor memory for the inhibitory avoidance task (see Collier and Sladek, this volume). We found a pronounced age-related decline in memory performance (mean step-through latency $\pm$ SEM; 5 m.o.:209.4 $\pm$ 36.9 sec., 24 m.o.:69.7 $\pm$ 23.9 sec.). Aged subjects hosting grafts containing NE neurons exhibited significantly better memory performance than their age-matched unoperated controls (24 m.o. + NE graft: 205.5 $\pm$ 59.8 sec.). Four of six aged animals hosting NE grafts exhibited optimal performance, failing to re-enter the shock chamber for the entire 5 minute retention test. Grafts of cerebellum had no therapeutic effect on the age-related memory deficit (24 m.o. + CBLM graft: 87.2 $\pm$ 53.7 sec.). Histofluorescence analysis of transplants confirmed the presence of NE-containing perikarya and processes in the grafts, and indications of integration with the host brain. The results suggest that declining NE system function in old age contributes to performance deficits in inhibitory avoidance memory tasks, and that replacement therapy with NE neuron-containing grafts has a normalizing influence. Supported by AG00847 (JRS), NS15109 (DMG), and MH08829 (TJC).

224.2 INCREASED NEOPHOBIA: BEHAVIORAL IDENTIFICATION OF A SUBPOPULATION OF MEMORY-IMPAIRED, NOREPINEPHRINE-DEFICIENT AGED F344 RATS. T.J. Collier and J.R. Sladek, Jr. Department of Anatomy, Univ. of Rochester Sch. of Med., Rochester, N.Y. 14642.

Behavioral studies of adult rats with lesions of the locus coeruleus-dorsal bundle norepinephrine (NE) pathway indicate that increased neophobia is a reliable correlate of damage to this system (Martin-Iverson et al., (1982), Pharmacol. Biochem. Behav., 17:639; Tombaugh et al., (1983), Brain Res., 261:231). Previous studies utilizing quantitative histofluorescence procedures indicate that NE content declines in locus coeruleus neurons as a function of aging in F344 rats (Sladek and Blanchard, (1981), In: "Brain Neurotransmitters and Receptors in Aging and Age-Related Disorders", S.J. Enna et al. (eds.), Raven Press, N.Y., p. 13). To determine whether this age-related decrease in locus coeruleus perikaryal fluorescence translates into behavioral change, we compared 4 month old (n=15) and 23 month old (n=47) F344 rats on a 2-bottle test of gustatory neophobia. Subjects were restricted to drinking water from 2 bottles over a daily 30 minute period for 5 consecutive days. On day 6, a bottle containing a 0.1% saccharin solution was substituted for one bottle. Gustatory neophobia was assessed by determining the amount of novel saccharin solution consumed relative to total fluid intake over a 30 minute test period. Rats consuming $<$ 50% of their total intake in the form of saccharin were defined as neophobic. Four month old subjects did not exhibit neophobia, consuming a mean of 57.8% of their fluid intake as palatable saccharin solution. In contrast, 23 month old subjects exhibited an increased tendency toward neophobia, consuming a mean of 45.9% of total intake as saccharin solution (t (60) = 3.2, $p < .01$). Not all aged animals were neophobic. Rather, the effect was characterized by a shifting of the old population toward greater incidence of neophobia: 20% (3 of 15) of the young animals consumed less than 50% of their intake in saccharin, 57% (27 of 47) of the old population fell in this category. Selected neophobic (n=12) and non-neophobic (n=7) aged subjects were also tested in a step-through inhibitory avoidance memory situation. These two groups of aged animals exhibited marked differences in memory performance 24 hr. after training. Neophobic aged animals showed poor retention (mean step-through latency $\pm$ sem: 69.7 $\pm$ 23.9 sec.) while non-neophobic aged animals performed significantly better (173.3 $\pm$ 38.4 sec.). These results suggest that gustatory neophobia may provide a simple behavioral index for identifying a subpopulation of memory-impaired, NE-deficient aged rats.

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224.3 EFFECT OF AGING ON Na-K ATPase ACTIVITY IN CEREBRAL MICROVESSELS, LEPTOMENINGES, CHOROID PLEXUS AND DURA MATER OF RAT. L.J. Embree, D.W. Jackson*, I.F. Roubein, Dept. of Neurology, Louisiana State Univ. Sch. of Med., Shreveport, LA 71130.

Maintenance of ionic homeostasis and active transport are essential for the functional integrity of neural tissues, and these fundamental biologic processes may be compromised to some degree in aging brain. In many tissues including brain the capacity for active transport of Na+ and K+ is directly proportional to the activity of Na-K ATPase, but information is lacking on age-related changes in the activity of this enzyme in neural tissues. Therefore this study of Na-K ATPase in four CNS related tissue preparations: cerebral microvessels (CMV), leptomeninges, including associated blood vessels (LM), choroid plexus (CP) and dura mater (DM), from young and aged rats was undertaken.

Male Sprague-Dawley rats (35-36 months and 3-4 months) were decapitated and dissection of the four specimens, including cerebral hemispheres from which microvessels would be isolated, was performed immediately in a chilled environment.

The technique of enzyme assay was a modification of Betz, et al. (Brain Res., 192:17 1980). Phosphorus analysis was by a microadaptation of the method of Martin and Doty, (Anal. Chem. 21:965 1949). Protein levels were determined by the Lowry method.

	Na-K ATPase Activity	
Tissue	Young (3-4 mo.)	Aged (35-36 mo.)
CMV	4.04 ± 0.47	2.64 ± 0.09*
LM	2.18 ± 0.21	1.50 ± 0.15*
CP	2.44 ± 0.34	2.98 ± 0.08
DM	0-0.27 (range)	0-0.17 (range)

Activity in μmoles Pi/mg protein/hour as Mean ± SEM (n=4). *Statistically significant ($p<0.05$).

These data show an age-related decrease in Na-K activated ATPase in CMV and LM. The reduction in this catalyst for energy conversion could be associated with an impairment of active transport and/or ionic homeostasis. Such an alteration in the capacity for active transport may be responsible for an insufficient response to the brain's energy and metabolic requirements, causing functional disturbances of neural tissues and producing neurologic symptomatology in the aged.

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224.4 A MODEL OF MULTINFARCT DEMENTIA: THE SPONTANEOUSLY HYPERTENSIVE RAT WITH SEVERE CEREBROVASCULAR LESIONS. G.Pepeu, F. Pedata*, F.Casamenti*, L.Bracco*, G.Spignoli*, S.Banfi* and L.Dorigotti*. Departments of Pharmacol. & Neurol. Florence Univ. 50100 Florence & I.S.F. Pharmacol.Lab. 20090 Trezzano sul Naviglio, Italy.

Spontaneously hypertensive rats (SHR) given 1% NaCl in their drinking water rapidly develop severe hypertension and cerebrovascular lesions (Okamoto, K. ed. Spontaneous hypertension, Springer Berlin, 1972). Three groups of male rats are used in the present experiments: 1) normotensive age matched rats Wistar Kyoto strain; 2) age matched SHR drinking tap water; 3) SHR drinking 1% NaCl for 14-22 weeks. At sacrifice blood pressure were respectively 125±3, 188±5, 213±4 mmHg. Macroscopic cerebrovascular lesions were present in 61% of the saline drinking SHR. No differences between the 3 groups were found in choline acetyltransferase activity measured in the cerebral cortex, hippocampus and striatum. In the saline drinking SHR high affinity choline uptake (HACU) rate was higher in the hippocampus (+18%, $P<0.05$) and striatum (+20%, $P<0.05$) than in normotensive rats. In the SHR drinking water HACU was higher than in the normotensive rats (+15%, P 0.05) in the hippocampus only. No difference was found in the cerebral cortex of the 3 groups. However ACh release from electrically stimulated cortical slices was lower at rest (-27%, $P<0.05$), 0.2 (-52%, $P<0.01$) and 1 (25%, $P<0.05$) Hz stimulation frequency in the SHR plus saline than in the other groups. The SHR plus saline showed and impairment in the acquisition of an active avoidance conditioned response (pole jumping) as indicated by a larger number of training sessions to reach criterion in comparison with the other groups. In a passive avoidance test 36% of the SHR plus saline and 12.5% of the SHR showed an acquisition impairment. In conclusion, SHR with cerebrovascular lesions show changes in brain cholinergic mechanism and a discrete cognitive impairment and could represent a suitable model of multinfarct dementia.

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224.5 UNCONDITIONED SOMATOMOTOR AND CARDIAC RESPONSES AS A FUNCTION OF AGE IN THE FISHER 344 RAT. Sheryl R. Ginn and Shirley L. Buchanan, WJB Dorn VA Hospital and Univ. of South Carolina, Columbia, SC 29201

The heart rate (HR) orienting response (OR), shock thresholds, and unconditioned HR responses (URs) to unsignalled shock were assessed in Fisher 344 rats of both sexes, aged 6, 12, 24 and 30 months. The HR OR was measured in response to 10 presentations of a 75 db, 4-sec tone. For half the animals of each age and sex, tone frequency was 10,000 Hz, and for the other half, 1200 Hz. Shock thresholds and unconditioned HR responses were assessed in response to 10 unsignalled presentations each of 6 shock intensities (0, .05, .1, .25, .5 and 1.0 mA, .25 sec duration) delivered in a random order to the pad of the right paw via a Grass Model E5H electrode. Leg flexion responses to the unsignalled shock were recorded by a Grass F.03 force displacement strain gauge transducer attached to the right hind paw. The shock intensity eliciting the following behaviors was recorded by an experienced observer: (a) a "twitch" of the leg without complete withdrawal; (b) a complete "flexion" of the leg without other bodily movement; and (c) a "jump", consisting of movement of the entire body.

The 30-mo old rats demonstrated higher thresholds than the other groups for the "jump" response only. Additionally, sex differences were found for all three responses, with males of all ages showing higher thresholds for the "twitch" and "flexion" responses, and lower thresholds for the "jump" response. The HR UR was in all cases an HR acceleration. The 30-mo old males showed smaller cardiac URs than did the younger males, but this difference was not found for the females. The cardiac component of the orienting reflex consisted of bradycardia in all animals. This decelerative HR response was larger for the 10,000 Hz than for the 1200 Hz tone. The 30 mo-old rats showed smaller ORs than the 12- or 24-mo olds, but the 6-mo old animals demonstrated much smaller ORs than the other groups, due to increases in movement in this group. Females showed larger cardiac ORs than males, particularly during the early trials. These data suggest that older males are generally less responsive than older females or younger males, which may contribute to previously reported performance deficits in various learning tasks in old males.

224.6 AN AGE COMPARISON OF SPATIAL FORGETTING AND THE DECAY OF LTE. C.A. Barnes and B.L. McNaughton, Behavioral Neuroscience Program, Dept. of Psych., Univ. of Colorado, Boulder, Colorado 80309.

The hypothesis that long-term enhancement (LTE) of hippocampal synaptic transmission is the mechanism by which spatial information is stored in the brain predicts a relationship between behavioral forgetting and the decay of LTE. While it is presently not possible to make the necessary comparison directly, the ability to induce LTE by electrical stimulation of perforant path fibers to the fascia dentata provides the opportunity for an indirect test of this prediction. In the present studies, this was carried out by comparing, in young (12 mo) and old (24 mo) rats, the exponential decay rates for spatial memory on a circular platform maze (involving escape from a brightly illuminated surface into a concealed goal tunnel) with the decay rates observed for perforant path LTE. While both phenomena were well described by exponential functions fit by least squares, it is clear from the fact that LTE decayed about twice as quickly as spatial memory (see table) that the simplest hypothesis of a direct linear relation is untenable. This is hardly surprising since most models of information storage would predict that performance would decay more slowly than the strength of the trace and also since the decay rates of both processes are affected by the parameters of the inputs which generate them. A stronger test can be made, however, if two groups of subjects can be shown to differ with respect to both the behavioral and the physiological rate constants (as has been shown here for the young and old animals). In this case, the hypothesis that LTE is the storage mechanism predicts that the between-group ratios for the behavioral and the physiological measures should correspond. In the present data the correspondence is a good one (about 9% difference). These findings thus provide further support for the hypothesis.

(Supported by PHS Grant AG-003376).

DECAY RATE ($Days^{-1}$)	Young	Old	Young/Old
SPATIAL MEMORY	0.014	0.030	0.47
LTE	0.027	0.062	0.43

224.7 AGED RHESUS MONKEYS (MACACA MULATTA) ARE MODERATELY IMPAIRED IN VISUAL RECOGNITION. S. K. Presty*, L. C. Cork*, D. L. Price, R. G. Struble, L. C. Walker, J. Bachevalier and M. Mishkin. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; Lab of Neuropsychology, NIMH, Bethesda, MD 20205.

A gradual decline in memory function is a prominent feature of aging in humans, and age-related memory deficits also occur in nonhuman primates. To determine whether this impairment in memory extends to recognition, perhaps the most fundamental form of memory, we trained monkeys of different ages on a delayed nonmatching-to-sample task using trial-unique stimuli. Animals were divided into three age groups of three to four monkeys each: Group Y (young, 3-6 years of age); Group I (intermediate, 14-17 years of age); and Group O (old, 27-30 years of age). On each trial, the animal was presented a sample object over a central food well and, ten seconds later, was given a choice between this sample and a novel object, each over a lateral well. The animal was rewarded for choosing the novel object. Twenty such trials were given daily until animals reached the criterion of 90 correct responses in 100 consecutive trials. Two weeks later, animals were retrained to criterion on this basic task and were then given a performance test in which delay periods were extended from 10 seconds to 30, 60, and 120 seconds, and list lengths were increased from one object to three, five, and, finally, ten objects. Although trials to learn and relearn the basic task tended to increase progressively with age, group differences were not significant. On the performance test, however, where memory demands were increased, there was a reliable and systematic decline in test scores with age: Group Y = 95%; Group I = 89%; and Group O = 83% ($p < 0.05$ for all comparisons). In the latter test, Group Y maintained a stable level of performance across all conditions, whereas Groups I and O did so only through list lengths of three, after which their scores showed a progressive decrease.

The moderate decline of visual recognition with increasing age, demonstrated here in monkeys, parallels the memory decline with advancing age observed in humans. The results provide additional support for the use of monkeys as an animal model for human aging. Further, since much of the neuroanatomical substrate of recognition memory in the monkey has been delineated, the substrate can now be investigated for evidence of increasing pathology with age.

224.8 THE EFFECT OF MSH/ACTH 4-10 ON DELAYED RESPONSE PERFROMANCE IN YOUNG AND AGED RATS: DIFFERENTIAL DOSE RESPONSES. B.A. Turnbull, L.H. Miller*, L.P. Taylor* and L.J. Traficante*. Department of Biobehavioral Sciences, Boston University Medical Center, Boston, MA 02118.

MSH/ACTH 4-10, at a dose of 95 ug/kg (IP), significantly improves performance by animals in a Hunter delayed response apparatus (Turnbull, B.A., et al., Pharmac. Biochem. Behav., in press). Recently, we found that MSH/ACTH 4-10 could reverse deficits in delayed response performance induced by anticholinergic agents (Turnbull, B.A., et al., presented: 14th CINP, Florence, Italy). The present study examines the effect of various doses of MSH/ACTH 4-10 on delayed response performance in animals when young and aged.

Long-Evans, male rats (n=6) received vehicle, MSH/ACTH 4-10 47, 95, 190 and 285 ug/kg (IP) 1 hr before behavioral assessment in the Hunter paradigm at ages 3 and 30 months. Delayed response performance was assessed at stimulus-response delay intervals of 0-12 seconds. Significant differences between age (0.0012) and treatments (0.0092) were found. Age/delay (0.0039) and age/treatment (0.0001) interactions were also noted. Animals 30 months old performed more poorly than when 3 months of age at the 8 and 10 second periods of delay when collapsed across treatments (0.05). Collapsing across delay periods, performance of younger animals was significantly more accurate than when aged when both received control (0.01), MSH/ACTH 4-10 47 ug/kg (0.01) and 95 ug/kg (0.01). No performance difference existed between young and aged animals when both received MSH/ACTH 4-10 at a dose of 190 ug/kg. Treatment with MSH/ACTH 4-10 285 ug/kg resulted in more accurate delayed response performance in animals when aged than when young (0.01). Examination of MSH/ACTH 4-10 dose response curves in the Hunter paradigm reveals an inverted "U" relationship for animals when young, and a positive linear dose response for animals when aged.

One possible explanation for the differential dose responses of MSH/ACTH 4-10 found in young and aged animals in this design is the functional age-related decrease in acetylcholine levels in the CNS of aged animals. The present findings, taken with past work implicating enhanced cholinergic transmission mediation of cognitive effects of MSH/ACTH peptide fragments, support the concept of aged-related decrease in neuronal ACh resulting in differential MSH/ACTH fragment dose response profiles between young and aged animals in cognitive paradigms such as the Hunter delayed response design.

224.9 OLD RATS DO NOT LEARN TASTE AVERSION AS WELL AS YOUNG RATS AFTER LONG CS-UCS DELAYS. Gregory N. Ervin*, Dana M. Vaughn and Barrett R. Cooper. (SPON: Warren C. Stern). Dept. Pharmacology, Wellcome Res. Labs, Res. Tri. Pk., N.C. 27709.

Fisher-344 rats, male retired-breeders of 10-11 months (mo) of age, were obtained at different times from Charles River Co. Some rats were group-housed until 24-30 mo of age (old rats). Others were group-housed until 12 mo of age (young rats). All rats were singly-housed one week before testing for taste aversion learning. Rats were first adapted to a daily, 2-hr drinking period by allowing them access to water from 0800-1000 hrs for 7 days. On the 8th day, all rats were offered only 0.25% sodium saccharin (SACC) for the first 30 min, then only water for 90 min. Old and young rats were divided into one of three groups. One group received intraperitoneal (i.p.) injections of 12.5 mg/kg l-5-hydroxytryptophan (l-5-HTP) 15 min after the end of the SACC drinking period. Another group received i.p. injections of the l-5-HTP vehicle alone (H_2O, double-distilled, pH 2.4; 2 ml/kg) 15 min after the SACC drinking period. Finally, a third group received i.p. injections of l-5-HTP 360 min after the SACC drinking period. The next day, rats were offered only water for 2 hrs. The following day was Test Day 1, and rats were offered only SACC for 30 min, then only water for 90 min. SACC intake on Test Day 1 is given in the following table:

Treatment (delay after SACC)	N	Test Day 1 ml SACC (±SEM)	%(±SEM) of age-matched controls
I. 12 Months old			
1. vehicle (15 min	11	10.6 ± 0.53	100.0 ± 5.01
2. l-5-HTP (15 min)	14	0.14 ± 0.10	1.35 ± 0.92
3. l-5-HTP (360 min)	13	1.15 ± 0.36	10.94 ± 3.37
II. 24-30 Months Old			
1. Vehcile (15 min)	7	10.7 ± 1.08	100.0 ± 10.13
2. l-5-HTP (15 min)	10	0.40 ± 0.22	3.74 ± 2.06
3. l-5-HTP (360 min)	12	3.00 ± 0.83	28.01 ± 7.71

When l-5-HTP treatment followed initial SACC consumption by 15 min, old rats learned taste aversion as well as young rats ($p<0.25$). However, when l-5-HTP treatment was delayed 360 min after SACC, old rats did not learn taste aversion as well as young rats ($p<0.05$), suggesting that the memory of a novel flavor necessary for taste aversion learning decays more rapidly with time in aged rats. This measure of memory in rats may be sensitive to treatments which alter memory in humans and may provide a useful model of certain age-dependent changes in memory.

224.10 CATECHOLAMINES AND COGNITION IN AGED MONKEYS: IMPROVEMENT IN DELAYED RESPONSE PERFORMANCE BY THE ALPHA-2 AGONIST, CLONIDINE. A.F.T Arnsten and P.S. Goldman-Rakic. Section of Neuroanatomy, Yale Medical School, New Haven, CT 06510.

The search for neural mechanisms underlying cognitive deterioration in the aged has centered on the cerebral cortex, particularly the frontal and other association cortices. Amongst the many neurochemical changes that have been noted in human neocortex is a depletion of catecholamines, with noradrenergic loss further exaggerated in Alzheimer's Disease. In aged nonhuman primates regional analysis revealed large (50%) decrements in CA levels, particularly in the prefrontal and temporal cortices (Goldman-Rakic and Brown, Neurosci. 6: 1981). To explore the hypothesis that loss of CA in the the prefrontal cortex (PFC) may contribute to the cognitive impairments displayed by aged monkeys, we examined whether CAergic drugs would improve the performance of aged rhesus monkeys on tasks sensitive to PFC damage. The results of this experiment were compared to our previous pharmacological study of young monkeys with cognitive deficits due to 6-OHDA-induced CA depletions of the PFC (Brozoski et al., Science 215: 1979). The aged monkeys were trained on a variable delayed response task, so that within each session performance could be observed at delays ranging from 0 sec to the delay which resulted in chance responding. Weekly drug treatment consisted of a single dose of drug and a placebo administered at least 2 days apart; examination of a drug continued until a dose-response curve with selected replications was completed. A variety of drugs which facilitate CA transmission were studied, many of which had improved the performance of the young, 6-OHDA-depleted monkeys. In the aged monkeys, drugs which indirectly facilitate CA transmission had inconsistent effects. However, the directly-acting alpha-2 agonist, clonidine, produced a dose-dependent improvement of delayed response performance in all aged monkeys tested. Dose-response profiles from the aged and the young, 6-OHDA-depleted animals suggest that clonidine's effects may involve actions at post-synaptic receptors. It is unlikely that these beneficial effects of clonidine were due to the drug's hypotensive or sedative properties, as neither propranolol or diazepam improved performance. These findings re-emphasize the involvement of catecholamines in cognitive function and further suggest that clonidine may be clinically useful as a treatment for age-related cognitive disorders.

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224.11 AF64A NEUROTOXICITY: BEHAVIORAL AND ELECTROPHYSIOLOGICAL ALTERATIONS IN RATS. E.Lehr*, F.-J.Kuhn*, D.H.Hinzen* (SPON: S. F. Zornetzer) Dept. of Pharmacology, Boehringer Ingelheim, D-6507 Ingelheim, FRG

Male rats (Chbb:Thom), 200-220 g, 3-4 months old, under barbiturate anaesthesia were stereotactically given an intracerebroventricular injection of 20 nmoles AF64A (5 µl). Five animals were implanted additionally with electrodes for recording cortical EEG and the EMG from neck muscles for sleep studies. Spontaneous unit activity, population spikes, frequency potentiation, and responses to microiontophoretic application of acetylcholine (ACh) were investigated in dorsal hippocampal pyramidal cells of rats anaesthetized with urethane. Responses were monitored in subfield CA1 via extracellular recording. Habituation to a novel environment was analyzed by activity records in photocell actometers. All testing was performed three months after AF64A administration.

All animals survived. REM sleep was reduced, REM latency increased as found in old animals. No measured parameters of single or multiple unit hippocampal responses were affected. Microiontophoretic application of ACh readily produced excitation of hippocampal pyramidal cells, and population spikes evoked by commissural stimulation were facilitated by ACh. AF64A was found to produce no significant effect on the magnitude of frequency potentiation. AF64A-rats showed higher irritability compared to controls. Their locomotor activity was double as high as that of non-treated animals. However, the relative locomotor activity decrease as a result of habituation did not differ markedly in both groups.

Locomotor activity, behavioral habituation, and neurophysiological responses of hippocampal pyramidal cells exhibit a pattern different from that found in old animals. However, REM sleep pattern of AF64A-rats resembles that of old animals (Lehr,E., Kuhn,F.-J., Hinzen,D.H., Gerontologist, 23:78, 1983). Our results indicate that even with the very low dose of AF64A injected and after three months latency distinct alterations in behavior and sleep are measurable. We conclude that the site of action of AF64A seems to be presynaptic in origin.

224.12 **COMPARISON OF THE EFFECTS OF NUCLEUS BASALIS, DIAGONAL BAND AND SEPTAL LESIONS ON LEARNING AND MEMORY IN THE SPRAGUE-DAWLEY RAT.** H.J. Altman, R.D. Crosland, D.J. Jenden & R. F. Berman. Lafayette Clinic, Detroit, MI, 48207; Dept. Pharmacology, UCLA, Los Angeles, CA, 90024; & Dept. Psychology, Wayne State University, Detroit, MI, 48202.

Old rats have been shown to exhibit impaired retention in one-trial inhibitory avoidance tasks (Bartus, et.al., 1981) and learning in highly complex serial discrimination tasks (e.g. Stone 14-unit T-maze, Goodrick, 1973). Such deficits have generally been attributed to an age-related deterioration in central cholinergic functioning. Consistent with this hypothesis is the observation that damage to the principal cholinergic projection to the frontal cortex (i.e. Nucleus basalis of Meynert, NbM) interferes with retention performance in a passive avoidance task (Altman, et.al., 1983). The purpose of the present series of experiments was to determine whether lesions of the NbM would also interfere with learning in the Stone 14-unit T-maze. An additional goal was to see what effects lesions to either the medial septum (MS) or diagonal band of Broca (DBB) (regions whose primary site of innervation appears to be the hippocampus) might have on learning and memory. Lesions were made in young male Sprague-Dawley rats by injecting 7.5ug in 0.5ul ibotenic acid (rate: 0.1ul/min.) bilaterally into either the NbM, MS or DBB. Groups of young sham-operated and vehicle-injected rats were also included. An old group of unoperated rats (24 mos.) were included to compare the performance of the three young groups against that of the old. Two weeks following surgery all animals were put on a restricted diet and reduced to 80% of their initial free feeding body weights. All animals were then trained (1 trial/day; 5 days/wk for 5 wks) in the Stone T-maze. Following completion of Stone maze training the animals were trained in a standard one-trial inhibitory avoidance task and tested for retention of the original avoidance habit 24 hours later. All of the animals were then sacrificed, their brains removed and the hippocampus, striatum and frontal cortex dissected out and assayed for CAT and AChE. The performance of the old animals was impaired in both tasks. Young animals with lesions in the NbM were impaired in the shock avoidance task but not in the Stone maze. Performance of animals with lesions in either the MS or DBB were unimpaired in either task. These results suggest that there may be a fair degree of specificity associated with the nature of the involvement of the structures examined with respect to their involvement in an animal's performance of these tasks.

224.13 INTEGRITY OF THE VESTIBULAR-OCULAR REFLEX IN ALZHEIMER'S DISEASE. V. LAKSHMINARAYANAN*, R. P. FRIEDLAND, E. Muller*, E. KOSS*, and L. STARK . Sch. of Optometry, Neurol. Div., U. C., Berkeley, CA. 94720; Dept. of Neurology, U.C., Davis, VAMC. Martinez, CA. 94553; and Donner Lab., U. C., Berkeley, CA. 94720.

The presence of a central cholinergic deficiency has been well established in Alzheimer's Disease (AD), but the effect of this deficit on brain functions aside from cognition have not received much attention. Evidence from pathological and chemical studies of AD brain as well as our increasing knowledge of cholinergic neuroanatomy suggests that certain brainstem functions may be disturbed in AD. In particular, cholinergic involvement in vestibular functions has been well established.

We are presently testing the vestibular-ocular reflex (VOR) and VOR suppression in both light and darkness in subjects with presumed AD. We measured eye movements using the infrared reflection technique and head movements with a low torque precision potentiometer. Subjects were instructed to maintain gaze on a stationary light-emitting diode (LED) while rotating their head. VOR suppression was tested by attaching an LED to the head with instructions to the subject to maintain fixation of the LED during head movement.

Studies have been performed in two subjects with mild-moderate Alzheimer-type dementia. The data were qualitatively normal in all four conditions when the subjects could maintain attention on the task. In the VOR suppression task the eye movement records were noisy, possibly due to attentional drifts. Quantitative measurements were precluded due to non-compliance with instructions.

These preliminary findings suggest that peripheral and central vestibular mechanisms may be intact in the early stages of Alzheimer's disease.

224.14 PRESERVATION OF ACCURATE SPATIAL MEMORY IN AGED RATS. W.W. Beatty, R.A. Bierley*, J.G. Boyd* and W.S. Maki*. Dept. of Psychology, North Dakota State Univ., Fargo, ND 58105.

Developmental studies using cross sectional designs indicate that spatial memory in old rats is less accurate than in younger animals. In a series of experiments that examined the effects of various drugs (scopolamine, propranolol, phentolamine, haloperidol, methysergide, naloxone, morphine and amphetamine) on the accuracy of spatial memory we employed the same animals. To our surprise the performance of these animals in the 8-arm maze did not decline with advancing age, even though the rats were 22 mo. old at the final drug test. To determine whether or not accurate spatial memory was preserved when the rats were unambiguously old they were not tested and remained drug-free until they were 26 mo. old. When retested at this age with a 5 hr. delay imposed between their 4th and 5th choices the rats averaged 96% correct on choices 5-8 which exceeded their performance at 6 mo. of age (88% correct) when they were first tested. The performance of the 26 mo. old rats was also better than that of a 5 mo. old comparison group which averaged 91% correct at the 5 hr. delay.

In a subsequent experiment using a cross sectional design we compared the performance of naive rats that were 3 or 22 months old at the start of training. The younger rats adapted to the maze and met the learning criterion when no delay was imposed more rapidly than the older animals. This replicates findings from other laboratories using different strains. These animals are currently being tested at longer retention intervals and these data will be reported at the meeting.

Although our old rats maintained highly accurate performance in the radial maze which they learned when they were young and practiced throughout their lives, they performed poorly on a different spatial task (a cross-maze) that they acquired when they were already aged. Taken together our findings demonstrate that spatial behavior does not inevitably deteriorate with aging in rats, although the capacity for learning new spatial problems is clearly impaired. The preservation of a well practiced skill in old rats may be likened to "crystallized" intelligence (i.e., the preservation of language and the application of knowledge to problem solution) displayed by elderly humans without serious brain pathology.

224.15 BEHAVIORAL AND NEUROPATHOLOGICAL EFFECTS OF DEFFERENTIAL DIETS IN 24 MONTH OLD C57Bl/6j MICE. Hasker P. DAvis and L. Trombetta*, Dept. of Psychology and Pharmaceutical Sciences, St. John's University, Jamaica, NY 11439.

It has been suggested that the cholinergic system may play a significant role in the memory decline that accompanies aging. This idea is supported by neurochemical and neuroanatomical evidence that demonstrate a correlation between cholinergic decline and impaired cognitive function. One investigative strategy that has been employed in animals to ameliorate this cognitive decline is differential dietary loading of choline. The present study reports behavioral and neuropathological effects of differential choline diets in mice at 24 months of age.

Retired breeder C57BL/6j male mice at 8-9 months of age were placed on either a choline enriched diet ($\approx$50mg choline/gm diet), choline control diet (1-2mg choline/gm diet) or choline deficient diet ($<$1mg choline/gm diet). An additional group of mice were placed on a choline enriched diet at 18 months of age after having been maintained on the choline diet from 8-9 months of age. At 24 months of age, mice were assessed for 7-day retention of one-trial passive avoidance training. Application of the Mann-Whitney U-test on step-through latencies at test indicated no significant difference between groups of mice started on differential diets at 8-9 months of age. Mice placed on a choline enriched diet at 18 months of age tended to perform better than the mice placed on differential diets at 8-9 months of age ($p < .10$).

Electron microscopic analysis of hippocampus revealed degeneration of astrocytes of animals on the choline deficient diet.

Mitochondrial enlargement was noted in both astrocytes and neurons. Large abnormal nonmembrane bound filamentous inclusions were observed in hippocampal neurons of mice on the choline deficient diet.

224.16 FAILURE OF OXOTREMORINE TO IMPROVE MEMORY IN RATS WITH LESIONS OF THE NUCLEUS BASALIS OF MEYNERT. R. F. Berman[1], R. Crosland[2], D. J. Jenden[2], & H. J. Altman[3], [1]Dept. of Psychology, Wayne State Univ., Detroit, MI 48202, [2]Dept. of Pharmacology & Brain Research Institute, UCLA, Los Angeles, CA 90024, [3]Lafayette Clinic, Detroit, MI 48207.

Loss of cholinergic function has been linked to cognitive deficits, including impaired memory and learning. Consistent with this view is the observation that destruction of the cells of origin of the major cholinergic projection to the forebrain (i.e., nucleus basalis of Meynert, NbM) in rats reduces neocortical levels of cholinergic markers (CAT & AChE) and impairs retention of shock-avoidance training. In the present studies we have attempted to improve retention of shock-avoidance training in NbM-lesioned rats by administering oxotremorine, a potent cholinomimetic, immediately after training or 30 min prior to retention testing. Two hundred and twenty-two adult, male Sprague-Dawley rats (300-350 g) were surgically anesthetized and stereotaxically injected bilaterally with the neurotoxin Ibotenic acid (7.5 ug in 0.5 ul artificial CSF as vehicle). An additional 20 animals were injected with vehicle only and 26 animals were sham-operated controls. Animals were allowed two weeks postsurgical recovery before training. Training consisted of placing rats into the lighted chamber of a two-chambered shuttle box, allowing them to enter the other darkened chamber, and then administering a 3 sec 1 mamp inescapable footshock. Animals were then removed from the apparatus and tested for retention of the footshock experience 24 hr later by again placing them into the lighted chamber and measuring their latency to enter the darkened chamber. As expected, sham-operated and vehicle-injected animals showed long latencies to enter the darkened compartment indicating good retention of the footshock experience. In contrast, rats with lesions of the NbM showed significantly shorter latencies to enter the darkened compartment compared to sham-operated and vehicle-injected animals indicating impaired retention. Injections of oxotremorine in doses of 10, 32, or 56 ug/kg, ip, immediately after footshock failed to improve memory tested 24 hr later. Similarly, injections of 1, 10, 32, 56, 72 or 100 ug/kg, ip, oxotremorine given 30 min prior to retention testing were also without effect. These results indicate that at the doses employed, and under the training conditions described, oxotremorine does not appear to be an effective agent for ameliorating retention deficits in NbM-lesioned rats.
(Supported by grants from the Wayne State University Neuroscience Program and NIA AG03571)

224.17 MHPG EXCRETION IN GERIATRIC PSYCHIATRIC PATIENTS. R.C. Young, G.S. Alexopoulos*, E. Kent*, C.A. Shamoian, J.J. Mann. Cornell University Medical College and Westchester Division, The New York Hospital, 21 Bloomingdale Road, White Plains, New York 10605.

Dysfunction of brain noradrenergic neurotransmission may play a role in the pathophysiology of both late life depression and senile dementia of Alzheimer type. 3-Methoxy-4-hydroxyphenethyleneglycol (MHPG) is a major metabolite of noradrenaline (NE). Urinary excretion of MHPG has been used as an index of brain and peripheral NE metabolism.

Geriatric psychiatric inpatients were studied who were $\geq$ 60 years of age and met Research Diagnostic Criteria and DSM III criteria for primary major depression (n=8) or DSM III criteria for primary degenerative dementia (n=3). Patients were excluded who were receiving drugs known to alter NE metabolism or were unable to cooperate with the procedure. Urine collections were performed during a drug washout period while subjects were on a caffeine-free and low tyramine diet. Specimens were chilled during collection, and sodium metabisulfite was added. MHPG was measured by gas chromatography-mass spectrometry using d_3-MHPG as internal standard. Creatinine was measured by spectrophotometry.

Urinary MHPG excretion varied about sixfold among the depressives. It ranged from 523 - 3,116 ug/24 h (mean 1,709 ug/24 h) or 0.84 - 4.28 ug/mg creatinine/24 h (mean 2.41 ug/mg/24 h). Values in individuals were comparable in serial 24 h determinations. In demented patients the MHPG excretion tended to be lower, ranging from 431 - 1,129 ug/24 h (mean 870 ug/24 h) or 0.88 - 3.76 ug/mg creatinine/24 h (mean 1.86 ug/mg/24 h). Urine creatinine excretion was similar in the two groups. Among the depressives, treatment for four weeks with the antidepressant nortriptyline, which inhibits NE reuptake, reduced MHPG excretion by 40-70% (n=3; $p < .001$).

These preliminary findings suggest that measurement of MHPG excretion may be useful in the investigation of NE metabolism in psychiatric disorders of late life. It may also be helpful in studying the treatment of these disorders.

Supported by the Department of Psychiatry, Cornell University Medical College, The Greenwall Foundation, and The Xerox Corporation. D_3-MHPG was the gift of S. Markey.

224.18 REDUCED CORTICAL CHOLINE ACETYLTRANSFERASE ACTIVITY AND IMPAIRED PASSIVE AVOIDANCE BEHAVIOR IN RATS AS A FUNCTION OF TIME AFTER LESIONING OF THE NUCLEUS BASALIS OF MEYNERT. R.D. Crosland, R.F. Berman, H.J. Altman, and D.J. Jenden. Pharmacology Dept., UCLA, Los Angeles, CA 90024; Psychology Dept., Wayne State Univ., Detroit, MI 48202; and Lafayette Clinic, Detroit, MI 48207.

Cortical choline acetyltransferase (CAT) is greatly reduced in people with senile dementia of the Alzheimer's type when compared to cortical CAT in age-matched normals. Recent evidence suggests that this reduction is caused by the loss of cholinergic neurons which project from the Nucleus basalis of Meynert (NbM) to the cortex. An analogous group of neurons exists in rats. Lesioning of the NbM in rats results in reduced cortical CAT activity and impaired passive avoidance behavior, suggesting that such lesioning may provide a useful model for studying impaired cognitive function. Young, male, Sprague-Dawley rats were stereotaxically injected (bilaterally) with 7.5 µg of ibotenic acid (in 0.5 µl) into the region of the NbM. Sham-operated rats served as controls. Each animal was placed into the lighted chamber of a two-chambered shuttle box, allowed to enter the larger darkened chamber, and given a 1.0 mA, 3 sec, inescapable footshock. Retention of footshock training was tested 24 hours later by measuring the animal's latency to again enter the darkened chamber. Following retention testing, animals were decapitated and their cortices dissected out for assay of CAT activity. Animals were trained and tested for retention of passive avoidance behavior and their cortical CAT activity assayed 6 days, 2 weeks, or 12 weeks after they were lesioned. Cortical CAT activity alone was determined in a group of animals 3 days after lesioning. When cortical CAT activity in lesioned animals is expressed as a percent of activity in sham-operated animals, the following values were found: 3 days, 87±4%; 6 days, 80±4%; 2 weeks, 70±2%; 12 weeks, 76±5%. Over a period of 2 weeks after lesioning, cortical CAT activity declined to its lowest value (70% of control) and remained low for up to 12 weeks. Lesioned animals also demonstrated significantly impaired shock avoidance behavior at 6 days, 2 weeks, and 12 weeks when compared to sham-operated controls. We have shown that lesioning of the NbM in rats leads to sustained losses of cortical CAT activity and passive avoidance behavior. Supported by the NIA (AGNS07351) and Neuroscience Small Grants Program, Sch. of Med., Wayne State Univ.

224.19 APOMORPHINE-INDUCED BEHAVIOR IN AGING C57BL/6J MICE. P.K. Randall and J.S. Randall. Physiology and Biophysics and Andrus Gerontology Ctr. U.S.C., Los Angeles, CA 90089

A detailed analysis was made of apomorphine-induced behavior in 5-, 11-, 18-, and 28-month old C57BL/6J mice. Behavior was observed for 30 sec every 6 min for a period of 1 hour and rated on a 7 point scale after administration of 0.25, 0.5, 1.0, 2.0, 4.0, or 8.0 mg/kg apomorphine hydrochloride.

With increasing age the magnitude of response was diminished at short intervals after the injection while it was drastically increased at longer intervals. Stereotype ratings fell exponentially with time following peak response for all ages with a progressive increase in apparent decay constant across ages (5 mo=8.5 min, 11 mo=12.2 min, 18 mo=18 min, and 28 mo=42 min). The time of peak response increased with dose of apomorphine and age, and was most pronounced with the more highly rated behaviors. ED50's for meeting or exceeding each of the ratings calculated from best fitting logistic functions at each observation time supported this conclusion. These data are consistent with previous reports of decline in both absorption (and/or accumulation in brain) and clearance of the drug in aging rodents.

224.20 RADIAL ARM MAZE PERFORMANCE AND LESIONS OF RAT BASAL FOREBRAIN. B.E. Lerer, J. Warner* and E. Friedman*. Depts. of Psychiatry and Pharmacology, New York University Medical Center, New York, NY 10016.

The magnocellular nuclei of the basal forebrain (MNBF), located ventral to the globus pallidus in the rat, provide extensive cholinergic innervation to cortex. The MNBF is analogous to the human nucleus basalis which has been implicated in the cholinergic and cognitive dysfunction of Alzheimer's Disease. We previously showed that MNBF lesions produced deficits in aversively and appetitively motivated behavioral tasks and concomitant deficits in cortical choline acetyltransferase (Lerer et al. Soc. Neurosci. Abstr. 9:97, 1983; Friedman et al. Pharmacol. Biochem. Behav. 19:309-312, 1983).

In the present study, MNBF-lesioned and sham-operated control rats were compared in acquisition and retention performance in an 8-arm radial maze. Arms were baited with sunflower seeds and extra-maze visual and auditory stimuli provided spatial orientation cues. A rat was placed in the maze and allowed 10 min to collect seeds. The optimum strategy was to visit each arm once and collect all 8 seeds within 8 choices. Criterion performance consisted of making at least 7 new choices within the first 8 choices on 5 consecutive days. Rats were tested for a minimum of 10 and a maximum of 30 days. Subjects were food-deprived for 23 hr prior to each daily session. Half the MNBF-lesioned and sham-control groups were pre-operatively trained to criterion performance (pre-trained). The remaining rats were not introduced to the maze until after recovery from surgery (post-trained). Testing was conducted no sooner than 20 days after surgery and not unless a rat weighed 20 g more than its pre-operative baseline weight.

The post-trained MNBF-lesioned rats learned to collect the seeds although they needed twice as many sessions as the controls did to acquire the task. As regards criterion performance, all post-trained controls reached criterion within 12 days (median = 10 days). The post-trained MNBF-lesioned rats were impaired in criterion acquisition; several rats never reached criterion before the 30 days cutoff (median = 26.5 days). The pre-trained MNBF-lesioned and sham-operated controls were generally unimpaired when tested post surgery.

These data suggest that retention of learning pre-operatively acquired remained relatively intact following MNBF lesions, whereas acquisition and retention of new skills were impaired after MNBF lesions. (Supported by NIMH fellowship 5T32MH15137 to B.L. and USPHS RSDA grant MH00208 to E.F.)

MUSCLE AND MUSCLE AFFERENTS

225.1 Primary afferents from distal forelimb muscles and dorsal root ganglia to the external cuneate nucleus in the cat. Jasmin, L.*, Bakker, D.A. and Courville, J. Centre de Recherche en Sciences Neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec.

Transport of horseradish peroxidase (HRP) was used to demonstrate the distribution in the external cuneate nucleus (ECN) of the cat of primary afferents originating from 1) paw and forearm muscles and 2) dorsal root ganglia. In the first instance, nerve branches were sectioned near their respective muscles. The proximal stumps were isolated with paraffin and exposed to a solution of 40% HRP for 4 hours. In a second group of cats, dorsal root ganglia C7 and C8 were injected with 3 to 8 µL of 40% HRP. After 3 days, the animals were sacrificed and the medullae were processed for HRP using tetramethylbenzydine. Paw and forearm muscle afferents terminate within the medial third of the ECN and extend along its caudal two thirds. Terminal labelling from individual muscles is distributed along well localized longitudinal columns. However, territories of distribution from neighboring muscles overlap widely. There is a topographical pattern whereby paw is represented most medially, along the border with the main cuneate nucleus and more proximal muscle afferents terminate more laterally. No terminal labelling was seen in other regions of the ECN receiving afferents from proximal limb, neck and thoracic muscles. Primary afferent fibers labelled by injecting the dorsal root ganglia of levels C8 and T1 demonstrate terminal territories which extend widely in the mediolateral direction as well as along the longitudinal axis. The distributions from these two ganglia largely overlapped and were densest over the territories corresponding to the distributions from distal forelimb muscles. Terminal labelling was consistently found, in the ventral parts of the main cuneate nucleus following HRP transport from nerves to the forelimb muscles. (Supported by a grant from the Canadian Medical Research Council).

225.2 PATTERNS OF MUSCLE AFFERENT TERMINATION IN THE EXTERNAL CUNEATE NUCLEUS OF THE CAT D.A. Bakker, J. Courville, F.J.R. Richmond and V.C. Abrahams. University of Montreal, Montreal, Quebec and Queen's University, Kingston, Ontario K7L 3N6.

The external cuneate nucleus (ECN) has long been known to relay proprioceptive information from the forelimb to the cerebellum. However, recent anatomical and electrophysiological studies in the cat (Bakker, Richmond and Abrahams, 1984, J. Comp. Neurol., in press; Murakami and Kato, 1983, Exp. Neurol. 79: 472-487) have shown that the ECN is also a target for primary afferent fibers from neck muscles. In this study, primary afferent projections to the ECN from different neck, shoulder and forelimb muscles were mapped using the method of transganglionic horseradish - peroxidase (HRP) transport. Nerves supplying each muscle were sectioned at their points of muscle entry. Their central ends were exposed to 40% HRP solution for 3-4 hours. After a 3 - day survival period, the medulla was removed and processed for HRP activity using tetramethylbenzidine as the chromogen agent.

Afferent fibers originating from different muscle groups were found to project to separate regions of the ECN. The caudal two-thirds of the ECN received projections from all muscle groups. Afferent terminations from forelimb muscles occupied discrete zones in medial regions, while those from shoulder muscles were found in central regions and those from neck muscles were confined to restricted ventrolateral regions of the ECN. A different pattern of terminal distribution was observed in the rostral one-third of the ECN. Here, dense terminal labelling from neck and shoulder muscles occupied medial parts of the nucleus. No labelling was observed in the rostral ECN following exposure of forelimb muscle nerves to HRP. The results show that primary afferent fibers serving different muscle groups have characteristic zones of termination in the ECN. The fact that much of the ECN is occupied by muscle afferent projections from the neck and shoulder suggests that the ECN may play a major role in transmission of sensory information from proximal as well as distal body musculature.
Supported by the MRC of Canada.

225.3 PROJECTIONS FROM THE EXTERNAL CUNEATE NUCLEUS TO THE CEREBELLUM. AN EXPERIMENTAL STUDY WITH RADIOACTIVE TRACERS. Courville, J., Bakker, D.A. and Jasmin, L.* Centre de Recherche en Sciences Neurologiques, Département de Physiologie, Université de Montréal, Montréal, Québec.

Previous anatomical studies by Grant (Exp. Neurol. 5: 179-195, 1962) have shown that the cuneato-cerebellar projection terminates as mossy fibers and is localized in ipsilateral lobules V and anterior part of lobule VI, in rostral and central folia of the paramedian lobule and in the posterior vermian lobule VIII. In the anterior lobe, these projections were observed to be dense in paramedian regions and lighter in the vermis. In the present study, this projection was reinvestigated in the cat with the method of radioactive aminoacid tracers and autoradiography. Injections of 0.3 to 0.8 µl of tritiated leucine (conc. 250 µCi/µl) were placed in the external cuneate nucleus under visual guidance. Survival periods of 2 to 6 days were utilized. Typical grain accumulations over mossy fiber rosettes and silver grain alignments over preterminal labelled fibers were found exclusively within the granular layer. The projection was abundant ipsilaterally in lobule V, in adjacent vermian parts of lobule VI in the depth of the primary fissure, also in lobule VIII and in the rostral and the central folia of the paramedian lobule. These sites correspond to those obtained with silver degeneration methods. In addition, moderately abundant projections were present in lobules IV, III and II and gradually diminished in rostral cerebellar levels. Light scattered projections were observed in lobulus simplex, Crus I and lobule IX. Contralaterally, projections were encountered in the same regions as on the ipsilateral side. Their densities were moderate to light and their distributions parallelled those of the ipsilateral projections. A striking feature was a distribution in sagittal strips over the vermian area, ipsi.- and contralaterally, up to a distance of about 1 mm. from the midline. Further laterally, the grain deposits appeared continuous. A less conspicuous banding pattern could be seen in lobule VIII. In the projection area, the silver grain accumulations were found in both superficial and deep regions of cortex. The present distribution is much more extensive than the "forelimb areas" in the anterior and posterior lobes although the dense projections correspond to those regions. (Supported by the Canadian Medical Research Council).

225.4 DEPENDENCE OF PRIMARY MUSCLE SPINDLE AFFERENTS' SENSITIVITY TO SMALL DISTURBANCES ON THE VELOCITY OF UNDERLYING LARGER MOVEMENTS. T.K.Baumann* and M. Hulliger* (SPON: R. Maurer), Brain Research Institute, University of Zurich, Switzerland

At constant mean muscle length, primary spindle afferents are extremely sensitive to minute sinusoidal stretches. It has been suggested (e.g. R.B. Stein, Physiol. Rev. 54, 1974) that Ia afferents might contribute to correction of movement irregularities by servo-like action. We have investigated to what extent the sensitivity of Ia afferents to small disturbances is maintained during concomitant large movement.

Responses of 17 primary spindle afferents to sinusoids (50, 100 and 1000 µm half-peak-to-peak (hpp) amplitude, frequency 1 Hz) superimposed on and synchronized with slow triangular movements (velocities ranging from 0.05 to 1.6 mm/s, 1.2 mm hpp, centered 3 mm below maximal physiological length (MPL)) of the deefferented soleus muscle were studied in 12 cats anesthetized with pentobarbital.

For extremely slow triangular movements (0.05 mm/s) Ia sensitivity to small disturbances (50 µm sinusoids) was as high (472.8 ± 260.9 1/s/mm) as for constant mean length controls (452 ± 185 1/s/mm). However, with increased velocity of the underlying triangular movement the sensitivity to sinusoids fell progressively along a smooth sigmoidal curve; reduction to one half occured at triangle velocities around 0.25 mm/s, 90% reduction (to 60.7 ± 35.6 1/s/mm) was seen at 1.6 mm/s. Analogous trends, only with slightly lower absolute values of sensitivity, were obtained with 100 µm sinusoids. In contrast, Ia sensitivity to sinusoids of larger amplitude (1000 µm hpp) was virtually independent of the velocity of the concomitant triangular movement and nearly identical to the sensitivity obtained with small (50 and 100 µm) sinusoids at the fastest triangle velocity. Thus, velocity dependent reduction of sensitivity is much more pronounced for small than for large disturbances.

The velocities of triangular stretch covered in this study were, for technical reasons, quite slow when compared to the highest velocities of muscle lenghtening attained during natural movements. Isolated measurements at 6.4 mm/s (using 4 Hz sinusoids) are however consistent with the idea that Ia sensitivity to movement disturbances remains low at faster velocities as well. This lower sensitivity clearly puts extra demands on the gain of the reflex pathways for servo-like correction of movement disturbances to be effective.

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225.5 FREEZE-FRACTURE OF MUSCLE SPINDLE SENSORY ENDINGS. D. C. Quick. Dept. of Anatomy, University of Minnesota, Minneapolis, MN 55455.

In muscle spindles, forces due to stretching are transmitted along intrafusal muscle fibers to the sensory endings and cause the endings to become depolarized. It is not certain which cellular elements are involved in the transmission of forces or in transduction of mechanical energy to electrical energy, but it appears that mechanical linkages must be present between muscle and nerve fibers, and that the transduction system must include the nerve membrane. A freeze-fracture study of muscle spindles was undertaken in hopes of revealing intramembranous specializations that might be associated with mechanically stable intracellular junctions or mechanically activated ion channels.

Cat tenuissimus muscles were fixed and muscle spindles were dissected out and glycerinated. Spindles were mounted in a freeze-fracture device such that the plane of fracture would travel across the intrafusal muscle fibers and their associated sensory endings. In electron microscopy of freeze-fracture replicas, no specialized intercellular junctions were observed between the nerve and muscle fibers, except for relatively rare cases in which a villus-like extension of the sarcolemma protruded into a matching invagination of the nerve ending membrane. The membranes of nerve-muscle interfaces did not appear to have a strikingly different structure from adjacent non-interface areas, and at the boundaries between interface and non-interface areas, no specialized structures or discontinuities of particle distributions were observed. The sizes and area densities of intramembranous particles were measured in sixteen categories of membrane fracture faces, classified as P or E fracture faces, neural or muscular membranes, interface or non-interface regions, and associated with nuclear bag or nuclear chain intrafusal fibers. This quantitative analysis revealed slight differences in some of the membrane categories but the possible relevance of those differences to the force transmission and transduction systems were not apparent. Extremely high densities of intramembranous particles (over 7500 per square micron) were found in some membrane categories, accounting for as much as 70% of the membrane surface area.

The findings support the hypothesis that depolarizing forces may be distributed to the nerve ending as a whole, rather than to discrete patches of the ending's membrane.

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225.6 SPINDLE ARCHITECTURE AND DISTRIBUTION IN THE PROXIMAL HINDLIMB MUSCULATURE OF THE TURTLE. J.W. Hermanson, P.R. Lennard, and J.P. Jackson*. Dept. of Biology, Emory Univ., Atlanta, GA 30322.

The mammalian intrafusal fiber represents a unique, readily distinguished fiber class exclusively associated with muscle spindles. In contrast, fibers in turtle muscle spindles seem to be drawn from a pool of very small diameter cells (VS) which are not exclusively associated with the spindle apparatus. These VS fibers course along superficial fascicles, often appearing as large bundles of cells. Groups of two to seven VS fibers become encapsulated into spindles along a part of the length of the VS bundles, while other VS fibers remain unencapsulated. The VS fibers exhibit either fast or slow profiles when stained for mATPase, and the profile of an individual fiber can change along its length. The VS fibers often travel to a position just below the superficial muscle fascia prior to encapsulation. Considerable fiber torsion occurs within capsular regions. Tandem spindles are common along the length of the bundles.

There is a characteristic distribution of spindles and VS fibers with respect to other fiber types in the turtle proximal hindlimb musculature. For example, in the ambiens muscle (knee extensor, hip adductor) the VS fibers and spindles are concentrated primarily in superficial fascicles which also contain many small slow/oxidative (SO) fibers. The SO fibers are statistically larger than the VS fibers. Few spindles and VS fibers occur in intermediate and deep fascicles. Intermediate fascicles contain a mosaic of large fast/glycolytic (FG) fibers, as well as fast/oxidative (FO) and two classes of fast/oxidative and glycolytic (FOG) fibers. Deep fascicles contain predominantly FG fibers. Fiber type distribution establishes a gradient of decreasing oxidative and increasing glycolytic capacity along the superficial to deep muscle axis.

The localization of spindles and VS bundles to oxidative portions of turtle muscle parallels the association of mammalian muscle spindles with oxidative muscle regions. We propose that the bundles of VS cells constitute a pool of specialized muscle fibers from which certain cells can be drawn to participate in sensory functions.

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225.7 MORPHOLOGY AND HISTOCHEMISTRY OF THE FACIAL MUSCLES OF MACACA FASCICULARIS. R.L. Sufit, G. Poulsen*, C. Welt and J.H. Abbs*. Dept. of Neurology and Speech Motor Control Labs., Univ. of Wisconsin, Madison, WI 53792.

Despite the importance of the facial muscles in primate communication, there is a paucity of information regarding their morphology and histochemistry. Generally, physiologic analyses have been based on the assumption that facial muscles are anatomically similar to limb or masticatory muscles. We have initiated a series of studies to delineate specific characteristics of the facial muscles and to compare these with features of limb and masticatory muscles in the same animals. Tissue from facial, masticatory, and limb muscles was dissected in reference to bony and perioral landmarks. The tissue was mounted on tragacanth gum and quick frozen at minus 160° C in liquid nitrogen cooled isopentane. Serial sections (8 μm) were processed for morphology and histochemistry using H&E, modified Gomori trichrome, NADH-tetrazolium reductase, myosin ATPase, reverse ATPase, PAS, Oil red O and Verhoeff Van Giesen methods.

The facial muscles differed from the limb and masseter muscles in several notable ways. In the facial muscles, connective tissue, composed of both collagen and elastin, was prominent both within and between muscle fascicles. Generally, the facial muscle fascicles and myofibers were smaller and less uniform than in limb muscles. The facial muscle fascicles also varied in the numbers of myofibers over the length of the muscle. Although the predominant fiber orientation in a given facial muscle was longitudinal, some fibers always were oriented in more than one direction.

No muscle spindles were seen in serial sectioning of 1800 μm in the orbicularis oris inferior, or in other facial muscles; muscle spindles were observed in masseter of the same animals using similar techniques. Differentiation of facial muscle fiber types was possible with both oxidative and ATPase stains. Both type 1 and type 2 fibers were present in the facial muscles, with the majority being type 2. However, in contrast to limb muscles, there was no differentiation into type 2A and type 2B with the ATPase after acid pre-incubation at pHs of 4.3, 4.5, 4.6, or 4.7. These data indicate that while general similarities exist between limb, masticatory and facial muscles there are significant morphological and histochemical differences which presumably reflect their differential functions. Research supported by grants from NIH (NS-13274 and HD-03352) and NSF (BNS-8021609).

225.8 CAN MUSCLE FIBER TYPE PROPORTIONS BE PREDICTED FROM SKELETAL FORM? M.R. Warner*, S.B. Boyd*, W.J. Gonyea, R.A. Finn* and W.H. Bell* (SPON: T. Cope). Dept. of Cell Biology and Div. Oral & Maxillofacial Surgery, Univ. Texas Health Science Center, Dallas, TX 75235.

The purpose of this study was to determine if the variation in human craniofacial morphology and masseter myofiber characteristics such as fiber type proportion and mean fiber area, are interrelated.

Twenty-six causcasions (13 males and 13 females, $\bar{x}$ age=24.8 yrs) exhibiting a large variation in size and shape of the facial skeleton were included in the study. Biopsy specimens (100-150 mg) were taken from the masseter at a standard anatomical location. To gain a better understanding of how the myofiber characteristics of the biospy site corresponded to the myofiber structural arrangement of the whole muscle, fresh whole masseters were obtained from six caucasion cadavers (3 males and 3 females, $\bar{x}$ age=32.5 yrs). Biopsy and whole muscle specimens were processed using standard histochemical techniques. Individual fibers were classified as either Type I or Type II and the distribution, mean fiber area and cross-sectional area of each fiber type were determined by computer analysis of photomicrographs. Lateral cephalograms were digitized and analyzed by computer methods to generate a set of skeletal morphometric and biomechanical variables. Multivariate statistics were used to determine if significant relationships exist between facial skeleton morphology and masseter muscle morphology.

Whole muscle specimens demonstrated the masseter to be a heterogenous muscle with an uneven distribution of the fiber types. Anteriorly, Type I fibers predominated in both the superficial ($\bar{x}$=50.1%) and the deep ($\bar{x}$=64.4%) aspects, which decreased to 28.1% and 44.4%, respectively, in the posterior region. The mean fiber area of the different fiber types varied also, with the Type I fibers generally larger than the Type II.

Type I fibers were also found to be larger than the Type II fibers in the biopsy specimens. Multivariate analysis revealed that the size of the Type I fibers were positively correlated (r=0.586, p=0.005) with the size of the mandible. Individuals with similar skull size but proportionally greater anterior facial length, exhibited larger Type II fibers (r=0.582, p=0.005) and a higher proportion of Type II fibers (r=0.745, p=0.0001).

The regional variation observed in the whole masseter specimens indicates that the masseter is designed to function in a complex manner, with functionally distinct subregions rather than as a single homogeneous unit. This investigation also suggests that the morphology and proportion of the masseter muscle units is interrelated with the morphology of the supporting facial skeleton.

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225.9 RECEPTORS SUBSERVING CONTROL OF REFLEX STIFFNESS IN HERMIT CRAB. William D. Chapple. Physiology Section. Biological Sciences Group. University of Connecticut, Storrs, CT. 06268.

Mechanoreceptors in the abdomen of the hermit crab, Pagurus pollicarus, were studied to determine their role in the stretch reflex mediating stiffness control. These receptors were provisionally identified as hypodermal receptors since removal of the cuticle did not abolish the stretch reflex and there are no known muscle receptor organs associated with this muscle. Single units in the right first root of the fourth abdominal ganglion responded to ramp stretch with a phasic discharge. The decay in frequency at the end of the ramp was fitted with a least squares sum of two exponentials with time constants of 0.5 s and 4.3 s. The receptors were activated by isometric activation of the muscles as well as stretch, indicating that they are in series with the muscle fibers. Comparison of peak frequencies under conditions of isometric activation and a stretch chosen to simulate the force profile generated by isometric muscle activation indicated that the receptors are more sensitive to muscle activation than stretch. Peak frequency was a function of both muscle activation and stretch indicating that the threshold of the receptors is reduced during muscle activation. These receptors are part of a reflex that can maintain constant reflex stiffness in response to disturbances during active movement as well as static posture.

226.1 REDUCED MEMBRANE EXCITATION AND PROPAGATION DURING FATIGUE DEPENDS ON THE DURATION OF FATIGUE AND THE INTRINSIC PROPERTIES OF THE MUSCLE. H.S. Milner-Brown* & R.G. Miller
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Muscle fatigue has been attributed to many factors including a decrease in phosphocreatine, a decrease in pH and impaired impulse propagation. There is still some disagreement on whether impulse propagation is impaired during fatigue; due in part to (1) the differing duration and degree of fatigue in different studies and (2) the inherent differences in the intrinsic properties of the different muscles that were investigated. The current study was undertaken to help resolve these controversies.

Evoked muscle compound potentials (MCP)(and later twitches) were recorded, both before and after fatigue, from the first dorsal interosseous, (FDI), adductor pollicis (AP) and anterior tibialis (AT) muscles following supramaximal ulnar and peroneal nerve stimulation respectively. The muscles were fatigued by maintaining maximum isometric, voluntary index finger abduction, thumb adduction or ankle dorsiflexion for durations of 1-5 min.. The mean % decrease in maximum force (FATIGUE INDEX), the mean decrease in amplitude and increase in duration of the MCP, as well as the % decrease in twitch tension are summarized below.

Fatigue (min)	Fatigue Index% FDI	AT	AP	%ΔAmplitude FDI	AT	AP	%ΔDuration FDI	AT	AP	%ΔTwitch Tension FDI	AT
1	38	25	22	-31	-7	0	+45	+18	0		
2	57	38	40	-34	-14	-5	+74	+19	+25		
5	75	65		-50	-31		+70	+50		-73	-55

After prolonged fatigue (3-5 min.) there was a reduction in both MCP amplitude and twitch tension, with similar time courses of recovery.

The conclusions from this study are as follows: (1) muscle membrane excitation and impulse propagation velocity are reduced during muscle fatigue and (2) the magnitude of this impairment depends both on the duration and degree of fatigue, as well as the instrinsic properties of the particular muscle.

226.2 QUANTITATIVE CORRELATION BETWEEN ELECTRICAL AND MECHANICAL ACTIVITY AND HIGH ENERGY PHOSPHATES DURING FATIGUE AND RECOVERY OF A HUMAN HAND MUSCLE. R.G. Miller, H.S. Milner-Brown*, R.B. Layzer,* D. Giannini,* T.L. James,* J. Murphy-Boesch* & M.W. Weiner. (SPON: P.R. Weinstein). Department of Neurology, Children's Hospital of San Francisco, San Francisco, CA 94118 and Departments of Neurology, Pharmaceutical Chemistry, Radiology and Medicine, University of California/San Francisco, San Francisco, California 94143

In order to investigate the mechanism of muscle fatigue and recovery 31P Nuclear Magnetic Resonance (NMR) measurements of the human adductor pollicis (AP) were correlated with electrical activity (EMG) and force of contraction. NMR measurements were made of healthy human subjects in a 9 cm bore, horizontally oriented magnet operating at 95.8 MHz for 31P. The probe was constructed so that the AP rested on a two-turn 1.5 cm diameter coil and the force of a sustained isometric maximum voluntary contraction of AP was measured using a force transducer. Electrical activity of muscle (surface rectified EMG) was recorded with electrodes placed on the skin over AP. Control 31P NMR spectra were obtained in one minute (60 one second pulses) with easily visible, well resolved peaks for the sugar phosphates of ATP, PCr and Pi. Control PCr/PCr + Pi was 0.9. Control pH was between 7.05 and 7.1. Neuromuscular efficiency was defined as the force/EMG ratio kg/mv during a brief, 50% of maximal contraction. Both NMR and Force/EMG measurements were made under resting conditions, during fatiguing muscle contractions and during recovery.

During a sustained fatiguing contraction maximum force fell by 80%, the PCr/PCr + Pi fell to .21 and pH fell to 6.3. At the end of the fatiguing contraction neuromuscular efficiency was reduced to 57% of control, and during recovery returned to control within four minutes. The time course of recovery for both PCr and pH was rapid and similar to the recovery of neuromuscular efficiency, although there was an initial fall of pH during the early phase of recovery.

These experiments are the first to directly correlate NMR measurements of high energy phosphate with electrical and mechanical activity of human muscle during fatigue and recovery. This approach should be useful for the investigation of mechanisms underlying muscle fatigue in normal subjects and in a variety of muscle diseases.

226.3 MEDIAN FREQUENCY OF THE MYOELECTRIC SIGNAL AS A FUNCTION OF ELECTRODE LOCATION. S.H. Roy* and C.J. De Luca.
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Localized muscle fatigue during sustained contractions may be measured by monitoring changes in the median frequency of the myoelectric (ME) power spectrum. Because this technique relies primarily on surface electrodes to detect the signal, attention must be given to the sensitivity of the power spectrum to the placement of the electrodes with respect to the innervation zone and tendinous portions of the muscle. The shape of the ME signal depends on the observation distance from the signal source, the direction of the signal and variations in the conducting properties of the muscle fiber.

The tibialis anterior muscle of six normal subjects was tested during short term and fatiguing isometric contractions sustained at a constant force of 20%MVC and 80%MVC. ME signals were detected at numerous sites along the length of the muscle by multiple surface electrodes. The innervation zone was determined by electrical stimulation and verified later by observing the region in which action potentials were inverted. The ME signals were analyzed by a computer controlled device called the Muscle Fatigue Monitor (MFM) and the initial value as well as the rate of change of the median frequency were determined.

Despite considerable variability in the location and number (1 or 2) of innervation zones, the highest values of the initial median frequency always occurred at the region of the innervation zone and decreased proportionally with distance from the innervation zone. When the tendinous portion of the muscle was approached the median frequency value increased. The force level of the contraction does not appear to effect this relationship except to uniformly increase the value of the median frequency. The rate of decrease of the median frequency during fatiguing (80%MVC) contractions was not sensitive to electrode location. These results are consistent with calculations which indicate that the superposition of action potentials in the vicinity of the innervation zone provides a relative increase of the high frequency components of the ME signal. Similarly,the "edge effect" generated by the tendons truncate the action potentials and relatively increase the high frequency components of the ME signal. The fact that no trends were noted in the rate of change of the median frequency is consistent with the notion that the spectral shift is related to changes in conduction velocity and modifications of the discharge statistics of the motor unit.

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226.4 MEASUREMENTS OF SPECIFIC TENSION IN SINGLE SOLEUS AND MEDIAL GASTROCNEMIUS MUSCLE FIBERS OF THE CAT. Sylvia M. Lucas, Robert L. Ruff, and Marc D. Binder. Dept. of Physiol. & Biophys., Univ. of Washington, Sch. of Med., Seattle, WA 98195.

Indirect estimates of specific tension (force/cross-sectional area) of type-identified muscle fibers have been made for several cat hindlimb muscles (McDonagh et al., J. Morphol. 166:217-230, 1980). Intriguingly, in each case, a 3 to 5 fold difference in the estimated specific tensions of slow-twitch vs. fast-twitch muscle fibers has emerged (e.g. 0.6 kg/cm^2 vs. 2.3 kg/cm^2 for Type S and Type F medial gastrocnemius (MG) muscle fibers, respectively; Burke and Tsairis, J. Physiol. 234:749-765, 1973).

In this study, we have attempted to determine whether different fiber types have characteristically distinct specific tensions by making direct measurements of the sizes of and forces produced by single fibers of the cat soleus and MG muscles. Our approach entailed dissecting out single fibers (5 mm lengths) whose sarcolemmas had been chemically removed using a 5 mM EGTA "skinning" solution (pCa 8), and then attaching the fibers directly to a photodiode force transducer. Each fiber was first placed in relaxing bathing solution (22 ± 1^{o}C;pH 7.0;pCa 8), its sarcomere length set at 2.7μm using its laser diffraction pattern, and its diameter measured using a calibrated gradicule of a microscope (±2μm). Subsequently, each fiber was transferred to a maximally activating bathing solution (pCa 3.6) where tension was measured at a sarcomere length which gave the maximal value.

For soleus muscle fibers, the mean cross-sectional area and mean specific tension values were $3459\pm201\mu m^2$ (SEM;n=45, range 1385-6648) and 2.24 ± 0.11 kg/cm^2 (n=25, range 1.19-3.39), respectively. For the MG fibers studied, mean cross-sectional area and mean specific tension values were $2934\pm119\mu m^2$ (n=83, range 1018-5542) and 2.46 ± 0.08 kg/cm^2 (n=56, range 1.05-4.47), respectively. A small but significant negative correlation ($p<0.01$) was found between cross-sectional area and specific tension of fibers in both muscles. In several experiments, we attempted to type-identify the single MG fibers using a standard actomyosin ATPase histochemical assay. Those fibers which appeared to be Type S had a mean specific tension value (n=9;2.43 ± 0.15 kg/cm^2) equivalent to that for fibers which appeared to be Type F (n=22;2.59 ± 0.16 kg/cm^2). Although our sample of type-identified MG fibers is quite small at present, all of our results suggest that previous indirect measures based upon motor unit analyses may have underestimated the specific tensions of Type S muscle fibers.

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226.5 PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF INDIVIDUAL MOTOR UNITS OF CAT MUSCLE. P.M. Nemeth, J.L. Park*, D.G. Stuart, R.M. Reinking, L. Rankin, S. Vanden-Noven and T.M. Hamm. Depts. of Neurology and Anatomy, Washington Univ. Med. Sch., St. Louis, MO 63110 and Dept. of Physiology, Univ. of Ariz. Health Sci. Center, Tucson, AZ 85724.

A quantitative approach is being used to assess the relationship between neuromechanical muscle physiology and biochemistry of energy metabolism. Following collection of physiological data on single motor units of the tibialis posterior muscle of adult cats and glycogen depletion of the constituent muscle unit, transverse sections of muscle were stained for periodic acid Schiff and myosin ATPase; alternate serial sections were lyophilized. Fibers of the muscle unit identified histochemically were dissected from the lyophilized sections and analyzed biochemically with microanalytical techniques.

Biochemical and physiological data were, thus, obtained on the same 4 fast-contracting fast-fatiguing motor units:

Table 1. Enzyme Activities in Motor Unit Fibers

Motor Unit No.	Adenylokinase	Lactate Dehydrogenase	Malate Dehydrogenase
1	140±7 (4.9)	234±6 (2.4)	3.33±.26 (7.7)
2	145±7 (4.6)	253±9 (3.7)	3.10±.18 (5.7)
3	150±7 (4.4)	160±7 (4.1)	4.47±.31 (6.9)
4	161±12 (7.6)	294±6 (2.1)	4.11±.34 (8.3)

Values are means±SD of 8 fibers (moles/kg dry wt/hr) with % coefficient of variation in parentheses

Table 2. Neuromechanical Properties of Motor Units

Motor Unit No.	Nerve Conduction Velocity (m/s)	Twitch Contraction Time (m/s)	Tetanic Tension (g)	Progressive Fatigue Index (t_2/t_0)
1	88.0	17.2	24.4	.058
2	64.4	26.8	81.3	.028
3	71.3	29.3	92.7	.044
4	80.2	27.8	67.8	.010

Low coefficients of variation in enzyme activities corroborate the earlier finding of biochemical uniformity (Nemeth, Pette, Vrbová, J. Physiol. 1981) now on enzymes representing different biochemical pathways.

The major profits of this study arise from analysis of motor units with a wide spectrum of characteristics to test for relationships between the physiological and biochemical variables and to determine the predictive value of these criteria in any resulting motor unit grouping.

226.6 THE EFFECTS OF CAGE SIZE ON THE FUNCTIONAL PROPERTIES OF RAT HINDLIMB MUSCLE. 1. FORCE AND FATIGABILITY. L.L. Rankin*, R.M. Enoka, K.A. Volz*, R.M. Reinking*, M.J. Joyner*, D.G. Stuart. Depts. of Physiology and Physical Education, Univ. of Arizona, Tucson, AZ 85724.

This project addressed the effects of usage on muscle properties with the assumption that motor-activity level is influenced by the extent of the physical environment.

SD weanling rats were raised for 98-155 days in either a small FDA-approved laboratory cage (2/cage) or one 476x larger (15/cage). Subsequently, some contractile, electrical, and fatigue characteristics were determined in vivo for extensor digitorum longus (EDL) and soleus (SOL), muscles characterized as predominately fast-contracting ("fiber-type" distribution approximately 3%-SO, 59%-FOG, 38%-FG) and slow-contracting (84%-SO, 16%-FOG), respectively (Ariano et al., J. Histochem. Cytochem. 21: 51, 1973). The results included (n = 7-12):

Selected Characteristics[1]	Large Cage EDL	Large Cage SOL	Small Cage EDL	Small Cage SOL
Force:				
Body wt. (N)	5.07±0.30		4.20±0.58*	
Muscle wt.[2]	0.49±0.06	0.52±0.10	0.56±0.08*	0.53±0.07
Muscle force[3]	6.53±1.85	4.59±0.68	5.62±2.01	3.58±1.11*
Fatigue test[4]:				
Initial force[5]	2.22±0.60	4.04±0.49	1.97±0.74	3.65±0.87
Max. force[5]	4.55±1.09	4.11±0.46	3.92±1.22	3.79±0.84
Fat. Resistance[6]	59±14	90±16	36±21*	69±32*
RT 50-20[7]: initial	7±2	41±14	7±2	42±9
(ms) 6 min	18±4	58±16	24±7*	77±24*

*$p < 0.05$ between large and small cage; [1] Mean ± SD; [2] Muscle weight relative to total body weight (mN/N); [3] Force (100 Hz stimulation) relative to normalized muscle weight in ^{2}(N/mN·N^{-1}); [4] 13 stimuli at 40 Hz repeated at 1/s for 6 min; [5] Normalized as in 3; [6] Force at 6 min relative to initial value (%); [7] Relaxation time (measured between 50% and 20% values of post-stimulus force).

These results indicate that cage size had an effect on both SOL and EDL. The effects on fatigue resistance and relaxation time were comparable for the two muscles. The changes in force and muscle weight, however, appeared to be muscle specific; the large-cage rats developed a greater maximum force in SOL and a lesser normalized muscle weight for EDL.

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226.7 THE EFFECTS OF CAGE SIZE ON THE FUNCTIONAL PROPERTIES OF RAT HINDLIMB MUSCLE. 2. EMG AND FATIGABILITY. R.M. Enoka, L.L. Rankin*, K.A. Volz*, R.M. Reinking*, M.J. Joyner*, and D.G. Stuart. Depts. of Physiology and Physical Education, Univ. of Arizona, Tucson, AZ 85724.

As an assessment of cage-size effects on the electromyogram (EMG), we have monitored the compound muscle action potential (AP) during a fatigue test in which the test muscle was activated by supramaximal intermittent stimulation of its nerve. Average measurements of the duration, area, "mean" amplitude, and normalized depolarization rate for the 13 APs within each train were obtained at selected intervals during the fatigue test (Stuart et al., Proc. XXIX IUPS, XV: 190, 1983). The test muscles (n = 3 for each group) were SOL and EDL of small- and large-cage reared rats.

These parameters were affected differently by fatigue:

1. AP duration increased during the fatigue test for all groups of rats. The changes exhibited by SOL were gradual, with final (6 min) values of 160 and 130% for the small- and large-cage groups, respectively, that were not significantly different. In contrast, EDL experienced a rapid increase in AP duration (to 210% of the initial value at 1 min) which the small-cage group maintained until the end of the test but which the large-cage group significantly ($p < 0.05$) decreased (to 145% at 2 min) and maintained at values comparable to those for SOL.
2. Although eventually AP area decreased for all groups, initially the area of the EDL APs increased in a manner somewhat parallel to the potentiation observed in the force response. Cage size did not significantly affect AP area but there were differences in area between the two muscles at 1, 2, 4, and 6 min: EDL = 62%, 23%; SOL = 106%, 80%; at 1 and 6 min, respectively.
3. "Mean" amplitude declined during the fatigue test for all 4 groups with significant differences at 1, 2, 4, and 6 min between the muscles (EDL = 18% and SOL = 68% at 6 min) but with no cage-size effect. However, the data do suggest a trend in which the large-cage rats maintained initial "mean" amplitude values longer into the test before they began to decline; EDL = 0.67 vs 0.17 min, SOL = 3.0 vs 1.5 min for the large- and small-cage groups, respectively.
4. Depolarization rate decreased within 40 s to its final value (60%) for EDL with no differences due to cage size. The decrease for SOL was more gradual and was significantly different between the small- (73%) and large- (90%) cage groups at 6 min.

These data suggest that this test produces precontractile fatigue in both SOL and EDL with a trend toward a greater cage-size effect (viz., Rankin et al., accompanying poster) in SOL.

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226.8 EFFECT OF "DISUSE" ON MAMMALIAN FAST-TWITCH MUSCLE: JOINT FIXATION COMPARED TO NEURALLY APPLIED TTX. D. St-Pierre*, and P. Gardiner (SPON: A. Peterson). Sciences de l'activité physique, Univ. de Montréal, Québec, H3C 3J7.

The effect of "disuse" on the functional properties of fast-twitch (FT) mammalian muscle is controversial, perhaps since the various "disuse" models reduce activity to different degrees, and may introduce factors other than reduced activity. Our goal was to compare the effects of "disuse" produced by neurally applied tetrodotoxin (TTX), and joint fixation on the atrophic and contractile responses of the rat gastrocnemius. TTX was delivered to the left sciatic nerve, similar to the technique described by Betz & Caldwell (J. Physiol., 1983) and the paralysis maintained for 2 weeks. In a separate group of rats left knee and ankle joints were fixed for 2 weeks according to Fournier et al. (Exp. Neurol., 1983).

Muscle weights and in situ contractile properties are summarized below. Joint fixation produced a decrease in muscle wet weight and absolute tetanic tension. The degree of atrophy was more severe with TTX-disuse and was accompanied by a decrease in tetanic tension per unit muscle weight (N/G). In addition, TTX-disuse resulted in an elevation of twitch: tetanic ratio, a prolonged twitch, and an increased degree of fusion at 50 Hz. The normalized maximal rate of tetanic tension development ($\%P_o$/msec) was highest in the TTX group. Fatigue index was unaffected by either condition. The data suggest that complete disuse of mammalian FT muscle causes atrophy, slowing of contractile speed and a loss in contractile strength per gram of tissue, and are consistent with a loss of sarcoplasmic reticulum function and of myofibrillar protein concentration with disuse.

	P_t (N/g)	P_o (N/g)	TPT plus $RT_{\frac{1}{2}}$(ms)	Max.Tet. dP/dt ($\%P_o$/ms)	$\%P_o$ at 50 Hz	L/R mus. weight
C	2.3	16.1	31.2	2.01	55.9	1.12
(n=7)	0.8	0.7	3.3	0.25	11.7	0.06
Fixed	2.9	15.7	30.0	2.40	70.1	0.94[a]
(n=6)	0.9	0.9	2.9	0.30	13.1	0.08
TTX	3.4[a]	10.0[ab]	39.4[ab]	3.16[a]	83.2[a]	0.62[ab]
(n=10)	0.6	2.0	4.8	1.14	8.0	0.11

[a]Significant difference from group C ($p<0.01$)
[b]Significant difference between groups TTX and fixed ($p<0.01$)

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226.9 PHYSIOLOGICAL PROPERTIES OF SINGLE MOTOR UNITS IN RAT PLANTARIS FOLLOWING PARTIAL DENERVATION. P.F. Gardiner, R. Michel*, A.E. Olha* and F. Pettigrew. Groupe en Recherche Neuromusculaire, Sciences de l'Activité Physique, Université de Montréal, Montréal, Québec, H3C 3J7.

Short term functional sprouting of motoneurones following muscle partial denervation (PD) has been demonstrated previously via force responses to stimulation of intact axons (Brown and Ironton, 1978; Gardiner et al, 1984). To complement previous studies, we proposed to describe contractile properties of single motor units (SMU) in rat plantaris 7 days following partial denervation (PD) at which time sprouting is incomplete, and reinnervation has not occurred. Male Fisher rats (180-240, n=8) had left hindlimbs partially denervated under Na barbital anesthesia, by surgical transection of L4 radicular nerve. Seven days later, isometric in situ contractile properties of SMU were recorded following ventral root filament isolation, and compared to controls (C). Sprouting was not complete by 7 days (indirect= 35-91% of direct stimulation-evoked force) Comparisons of SMU twitch tensions (Pt) and fatigue characteristics (FI, Reinking et al. 1975) are summarized below. Only fast-twitch SMU (exhibiting "sag" on unfused tetanus, and post-tetanic potentiation), which constituted the majority (>90%) of SMU, are reported. Mean Pt was slightly (12%) increased after PD, due to increased proportions of SMU in the 75-300mN/g tension categories. Fatigue resistance was higher (i.e., lower FI) in PD units, especially those generating higher tensions, despite the fact that 44% of PD units demonstrated loss of EMG near the end of the fatigue regimen (15% in C). Following partial denervation, plantaris SMU become larger, more fatigue resistant, and demonstrate neuromuscular failure with trains of 40 Hz. The absence of very large (Pt>375mN/g) SMU in PD animals may represent their inability to expand their functional field in this short period following PD, as suggested by Slack and Hopkins (1982). (Supported by NSERC and MRC Canada)

Pt (mN/g):	0-75	76-150	151-225	226-300	301-375	376-450
C(n=79)	39.7	25.6	8.7	14.1	7.6	3.8
FI	(.57)	(.65)	(.71)	(.70)	(.76)	(.72)
PD(n=29)	22.7	37.1	18.5	18.5	3.7	-----
FI	(.56)	(.62)	(.62)	(.62)	(.60)	-----

Values represent % of sample in each tension category, with mean fatigue index (FI) in parentheses below.

226.10 EFFECT OF VARIED INTER-PULSE INTERVALS ON EVOKED HUMAN MUSCLE CONTRACTIONS. A.A. Vandervoort*, J.G. Quinlan* and A.J. McComas Departments of Neurosciences and Medicine, McMaster University Medical Centre, Hamilton, Ontario Canada L8N 3Z5.

Rapidity of muscle tension development may be a critical factor in some movement patterns and it has been shown that the force output of single mammalian motor units can be greatly enhanced by two closely-spaced stimuli in a tetanic train (Burke, R.E. et al., Science, 168: 122-124, 1970). We have now investigated the importance of this "catch" phenomenon at the beginning of contraction in two human muscle groups, the ankle dorsiflexors (DF) and plantarflexors (PF). With the use of a foot-holder designed to measure isometric twitch torques (Marsh, E., et al., J. Appl. Physiol: Respirat. Environ. Exercise Physiol 51: 160-167, 1981) responses of resting muscle to paired supramaximal stimuli of varied interpulse intervals (IPIs) were compared in five young adult volunteers. Two stimuli with a 1 millisecond IPI had no effect on the peak twitch tension (P_t). However, the response to paired stimuli separated by 4 ms was larger than the twitch evoked by a single stimulus, the respective mean values being 2.57 $\pm$ 0.13 for DF and 1.83 $\pm$ 0.11 for PF($p<.01$). Third and fourth stimuli, delivered with the same IPI (4ms), generated further, but smaller, increments in tension. Paired stimuli, separated by 4 ms, also had the effect of prolonging the contraction time (CT), the respective factors being 1.34 $\pm$ 0.07 for DF and 1.17 $\pm$ 0.06 for PF ($p<.01$). In absolute terms, the increases in CT were several times greater than the IPI. Increases in IPI beyond 4 ms resulted in a reversion of DF P_t to the baseline resting value, but for the PF muscle, this reversion did not begin until after 100 ms IPI. At long IPIs of 300 to 500 ms, slight depression of P_t relative to baseline was observed. High-frequency doublet firing of motor neurones would thus contribute substantially to rapid tension development in these muscles, with a relatively greater response in the DF group. The "catch" effect could be utilized in the programming of neuromuscular stimulators.

Supported by the Muscular Dystrophy Association of Canada. A.A. V. is a Research Fellow of the Gerontology Research Council of Ontario.

226.11 HUMAN PERIORAL MUSCULATURE: NATURAL HISTORY OF A MULTIPLE FORCE VECTOR MUSCLE COMPLEX. C. Blair and E. Müller.* Waisman Center, Univ. of Wisc., Madison, WI, 53706

In preparation for analyses of motor unit control during speech movements, we have done several descriptive studies of the perioral musculature, particularly the lower lip muscle Orbicularis oris inferior (OOI). A summary of these data will be presented:

Reconstructions from serial sections show that the muscle fibers in OOI are grouped into small fascicles (4-50 fibers) which often are surrounded by substantial amounts of fat and connective tissue. The majority of the fascicles are parallel to the vermillion border, but there are a considerable number in other planes.

In normal, healthy, young adults, maximal modiolar isometric forces ranged from 650 - 900 gm. The mean peak dF/dt and d^2F/dt ranged from 2 - 19 and .4 - 3.4 gm, and the time of these peaks with respect to the onset of the changes in force ranged from 66 - 94 and 31 - 69 msec.

The gross EMG activity of each of 6 perioral muscles during static and phasic isometric tasks was highly correlated among muscles and with force, even between muscles that are traditionally considered to be antagonists.

Endurance of the system was examined during static and phasic isometric contractions. Peak and the time of peak dF/dt and d^2F/dt were unchanged by prolonged static "conditioning" contractions, but the amplitude of the rapid contractions decreased, the duration increased, and the occurance of double peaks in force increased. Examination of EMG/force relationships with repeated trial, time series, multiple regression analysis and spectral analysis of the EMG suggest that much of the fatigue induced by these conditions is neural. There was no evidence of fatigue in the normal range of adult muscle use.

Observations of OOI motor unit activity during static and phasic isometric contractions include: 1. high interspike interval(isi) variability at constant forces, 2. small increases or decreases in mean isi with increases in force, 3. recruitment of m.u.s up to at least 85% maximal force, 4. spike-triggered averaged twitch tensions ranging from -1.4 - 4.7 gm, and 5. twitch times to peak from 10.9 - 90 msec. 6. Some units could not be activated voluntarily at any level of static force.

Supported by USPHS Grants NS00770 and NS13274. Computor software by P. Peterson, E. Müller, and G. McLeod.

226.12 ANALYSIS OF ELECTROMYOGRAPHIC SIGNALS AT VARIOUS SUSTAINED ISOMETRIC BITE FORCE LEVELS. G.T. Clark* and M.C. Carter (SPON: W.D. McCall) School of Dentistry, University of California, Los Angeles, CA 90024, USA.

Previous research on the jaw closing muscles has failed to show a decay in force during a sustained isometric clenching task when assessed with brief intermittant maximum voluntary efforts (Clark and Carter, J. Dent. Res. 61:257, 1982). This pattern is distinctly different from brief maximum voluntary force patterns observed in the limb musculature under fatiguing isometric conditions. Because of these differences and since comparative analysis for fatigue interaction between force and EMG activity has not been widely performed for the jaw, this study was undertaken.

Maximum voluntary bite force (MVBF) was measured on seven healthy male subjects using an intraoral force transducer. Subjects randomly sustained various force levels until pain tolerance was reached. Electromyographic (EMG) activity was recorded from the right anterior temporalis and superficial masseter using bipolar surface electrodes. The first and last 15 seconds of the sustained effort task were compared by measurement of the mean amplitude via digitized polygraph records from rectified integrated EMG and DC force level records. In addition, determination of the mean peak frequency for five 1 second intervals during both the first and last 15 seconds of the 50% isometric task was performed on 4 subjects using spectral analysis of the EMG signal.

The mean amplitude of the rectified EMG showed no significant change between the first and last 15 seconds. A comparison of the EMG/bite force amplitude ratio for the same periods also demonstrated no change. These results were consistent for all isometric force levels tested (25, 50,75 and 100%). Finally the results of the spectral analysis demonstrate that the mean peak frequency generally shifts to lower values when the first and last 15 seconds of the 50% isometric clenching task are compared.

The typical EMG frequency change characteristics thought to be associated with neuromuscular fatigue were present. The bite force and EMG amplitude data, however, did not show typical fatigue induced changes as seen in other motor systems. These results suggest a relative resistence of the jaw closers to neuromuscular fatigue with a sustained isometric task and question the use of EMG criteria alone as a fatigue index.

This research was supported by NIDR Grant No. DE06665.

226.13 **THE EFFECT OF MALNUTRITION ON DIAPHRAGMATIC CONTRACTILITY.** M.I. Lewis*, G.C. Sieck, M. Fournier and M.J. Belman (SPON:D. A. McAfee) City of Hope Medical Center, Duarte, CA 91010.

Malnutrition (MN) is a complicating factor in a number of respiratory diseases. The purpose of this study was to assess the influence of MN on the contractile properties of the diaphragm in 18 adult male Sprague Dawley rats. The rats were randomly divided into a control group (CTL) fed food and water ad lib, or a nutritionally deprived group (ND) presented water ad lib but fed only 1/5 of their estimated daily food requirement. This feeding paradigm was continued until the body weights of the ND animals had fallen to approximately 50% of the CTL group (ND: 233.1 ± 22.1g, vs. CTL: 468.2 ± 42.7g). Isometric contractile properties of uniform isolated strips of diaphragm were then studied in vitro. The phrenic nerve was stimulated supramaximally and fiber length was adjusted until optimal length (Lo) was set. Force was then measured at stimulation frequencies of 5,10,20,30,40,50,80 and 100 Hz with 2 min rests between each stimulus train. Fatiguability was assessed after continueous stimulation at either 20 or 100 Hz. Fatigue was arbitrarily defined as a force decrement of 50% of the initial tension for that frequency. The time taken to achieve this end point was determined and defined as the 'endurance time'. The effect of high (HF) and low frequency (LF) fatigue on the force frequency curve was assessed immediately after stimulation. Absolute tensions were dramatically reduced at all frequencies of stimulation in the ND animals (twitch tension-CTL: 35.5 ± 8.3g; ND: 17.7 ± 8.7g; peak tetanic tension - CTL: 100.5 ± 28.3g; ND: 41.0 ± 21.4g). The time to peak tension and the half relaxation time did not differ between the ND and CTL groups. The force frequency curves (FFC) were normalized to percent of maximal tetanic tension. Although the relative tensions at low frequencies were slightly greater in the ND group, the overall FFC's were not dissimilar. Both LF and HF fatigue caused the FFC to shift downward in both groups. However, this fatigue-related shift in the FFC was markedly attenuated in the ND animals. The endurance times in both HF and LF fatigue were significantly longer in the ND animals (HF: ND = 6.04 ± 1.7 sec; CTL = 2.48 ± 0.7 sec; LF: ND = 56.07 ± 12.9; CTL = 42.8 ± 3.5). These data indicate that MN dramatically affects the contractile properties of the diaphragm with the most striking effect being a marked reduction in diaphragmatic strength. Although endurance to fatigue appeared to be improved in the ND animals, this should be taken in the context of the severely diminished diaphragmatic forces in these animals.

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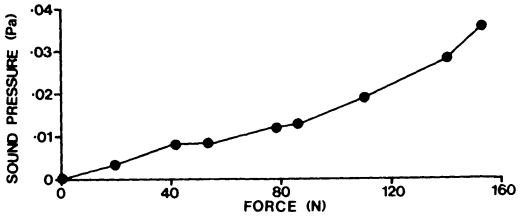
226.14 **CO-VARIATION OF FORCE AND SOUND RECORDED FROM THE HUMAN BICEPS MUSCLE.** L.A.Jones, I.W.Hunter and J.S.Outerbridge. Dept. of Otolaryngology and Biomedical Engineering Unit, Fac. of Med., McGill Univ., Montreal, Canada H3G 1Y6.

There has been a recent revival of interest in sounds recorded over the muscle belly during skeletal muscle contractions. These sounds have been reported to have a peak frequency of 25 Hz, and are therefore inaudible to the human ear. The objective of this study was to quantify the relation between muscle force and these sounds.

Subjects were positioned in an experimental rig so that the angle between the forearm and upper arm was 90 degrees. The forces resulting from the isometric contraction of the elbow flexor muscles were recorded at the wrist. A Bruel & Kjaer capacitor microphone (flat frequency response from 2 to 200 Hz) was affixed over the belly of the biceps brachii muscle (6 mm above the skin surface). Subjects produced forces ranging from 0% to 80% (in 10% increments) of their maximum voluntary contraction (MVC). The target forces were presented in a random order on an oscilloscope and subjects were required to match these forces and to maintain them at a constant level for 14 seconds. A rest period of 45 seconds separated each contraction. The output of the sound and force amplifiers was low-pass filtered (8-pole, cutoff 125 Hz) prior to 500 Hz sampling.

The mean force and absolute sound pressure (corrected for ambient noise) were calculated at each % MVC level. It was found that the relation between force and sound pressure was monotonic up to forces of approximately 160 Newtons. At higher force levels the sound pressure did not systematically increase. Analysis of the sound spectra indicated that there was considerable inter-subject variation over the 0 to 80% MVC range investigated. The previously reported peak at 25 Hz was not a consistent feature of the spectra at varying force levels. An example of the relation between force and sound pressure is shown.



MUSCLE II

227.1 **cAMP DECREASES THE Ca-SENSITIVITY OF EDTA-SKINNED BUNDLES FROM MYTILUS' ANTERIOR BYSSUS RETRACTOR MUSCLE (ABRM).** P. B. Chase* and B. C. Abbott. Dept. of Biol. Sci., Neurobiol. Sec., Univ. Sou. Cal., L.A., CA 90089-0371.

In "skinned" ABRM fiber bundles, 10μM cAMP increases the rate of relaxation in low [Ca^{++}] (F. Cornelius (1982) J. Gen. Physiol. 79:821; G. Marchand-Dumont and F. Baguet (1975) Pflügers Arch. 354:87). This is thought to be equivalent to release of "catch" by 5-HT. Our experiments confirm this observation when EDTA and EGTA (10mM each, 50mM BES, pH7.0) are used to make the plasma membrane permeable to small molecules. Contrary to the published results, cAMP does not increase the relaxation rate of detergent skinned bundles; they relax at a maximal rate in pCa8/0cAMP solution.

At least part of this observation can be explained by the reversible decrease of Ca-sensitivity by cAMP in EDTA skinned bundles; Ca-sensitivity of detergent (0.1% saponin) skinned bundles also decreases in cAMP, but this effect is not reversible and occurs along with a slow decrease observed in the absense of exogenous cAMP. EDTA skinned bundles also show hysteresis of the force-pCa relation (Ca-sensitivity of some muscles is increased after maximal stimulation; E.B. Ridgway et al. (1983) Science 219:1075); detergent skinned bundles do not show hysteresis.

The increase in sensitivity is dependent on the presence of membranes; we consider 2 possible roles for membranes: the responsible factor is either (i) an integral part of a membrane or (ii) soluble, but unable to diffuse out of the bundle because it is large relative to the small lesions caused by EDTA skinning. Some of the differences between EDTA and detergent skinned bundles may be explained by this lower permeability; intact Ca-releasing/sequestering structures (only in the EDTA skinned bundles) may overcome the 10mM EGTA Ca-buffer.

We conclude that cAMP controls relaxation of "lightly" skinned ABRM bundles by decreasing the Ca-sensitivity of the contractile filaments; it is probable that Ca-sequestering/release is also altered by cAMP. The presence of a factor which increases Ca-sensitivity is suggested; it is probably controlled by Ca^{++}. These mechanisms, althouth not understood in complete detail, could be responsible for control of catch in the intact muscle.

Supported in part by a USC Pre-doctoral Fellowship and a Grant-in-Aid of Research from Sigma Xi, the Research Society.

227.2 **CALCIUM SENSITIVITY OF MUSCLE FIBERS FROM TWO PREDOMINANTLY SLOW-TWITCH RAT MUSCLES.** B.L. Laszewski. Dept. Physiology and Biophysics, SJ-40, University of Washington, Seattle, WA 98195

Histochemical analysis of muscle fibers often correlates with the physiological properties of the muscle fibers. For example, most fibers from the rat extensor digitorum longus (EDL) muscle have high myosin ATPase activity (determined histochemically), brief contraction times, and low Ca sensitivity. On the other hand, muscle fibers from the rat soleus muscle, which histochemically show lower ATPase activity, have low maximum shortening velocities as well as high Ca sensitivity. However, there is not always a one-to-one correlation between the histochemical findings and Ca sensitivity as shown by Zeman and Wood (J. Histochem. Cytochem. 28:714-16, 1980) where both the fast fibers and the few slow fibers (determined histochemically) found in the predominantly fast-twitch EDL showed lower Ca sensitivity than slow fibers from the predominantly slow soleus muscle (in the rat).

This finding raises the following question: Are the fibers from the soleus muscle unique, or do fibers from other muscles also show high Ca sensitivity as compared with fast fibers or slow fibers from the EDL?

To answer this question, I looked at the Ca sensitivity of isolated, chemically-skinned single fibers from the predominantly slow-twitch rat adductor longus (88% SO, 12% FOG) and soleus muscles (84% SO, 16% FOG [Ariano et al, J. Histochem. Cytochem. 21:51-55, 1973]). Whole muscles were skinned for 1 to 2 days in 5 mM EGTA solution; single fibers were isolated and attached to hooks connected to a force transducer. Tension was recorded while bathing the fiber in various Ca concentrations. Solutions were buffered with EGTA resulting in free [Ca^{++}] ranging from 10^{-8}M to 10^{-4}M (pCa=8 to 4). Both the soleus and adductor longus muscle fibers had the same percent maximum force production at every Ca concentration studied. The Ca threshold (pCa ≈ 6.4), Hill fit (pK=6.08, n=2.2) and maximum force development (at pCa≈5.2) was identical for both muscle fibers.

Therefore, muscle fibers from the soleus muscle are not unique, but have Ca sensitivities similar to another predominantly slow-twitch muscle, the adductor longus. These results do not, however, answer the question of whether or not slow fibers from mixed muscles have different Ca sensitivities compared with slow fibers from predominantly slow muscles. Experiments to answer this question are underway.

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227.3 CALCIUM PARADOX IN SKELETAL MUSCLES: PHYSIOLOGICAL OBSERVATIONS M. Soza,* G. Karpati, S. Carpenter* Montreal Neurological Institute, Montreal, Quebec, Canada H3A 2B4

A major reduction of extracellular Ca^{2+} concentration for a brief period deprives the sarcolemma of its normal ability to prevent a massive lethal influx of Ca^{2+} into muscle fibers when normal extracellular Ca^{2+} concentration is subsequently restored. This phenomenon, called calcium paradox (CP), was first detected in cardiac muscle. We have produced CP in skeletal muscles. Rat hemidiaphragms were removed and placed in Ca^{2+}-free, oxygenated, 37°C Krebs' solution (KS) containing 15mM edetic acid (EDTA) for 15 minutes (phase 1) and then into regular KS for 20 minutes (phase 2). Microscopic examination after phase 1 shows separation of basal lamina from plasma membrane of muscle fibers. During phase 2, necrosis of muscle fibers sets in. Isometric tension of curarized hemidiaphragms was recorded in vitro at 37°C during phase 1 and 2 of CP. In our experiments, maximum twitch tension to supramaximal stimuli at 0.2Hz in regular KS is stable for long periods. Upon placing the muscle in Ca^{2+}-free KS with 15mM EDTA, a marked transient (2 min) contracture developed, associated with concomitant temporary (1 min) potentiation of twitch tension. After this, the twitch tension rapidly declined and was lost within 5 minutes. The resting tension remained stable near control levels. After 15 minutes, the KS with EDTA was removed and regular KS replaced. Within 2 minutes, a steady marked and sustained rise of tension (contracture) developed, while the twitch response did not return. Dantrolene sodium (1mM) or KCl (50mM) abolished the contracture and twitch potention of phase 1, but not the contracture of phase 2. Verapamil (50μM) abolished the contracture of both phase 1 and phase 2. 15mM Ca^{2+}EDTA, in regular KS, caused no physiological or microscopic changes of phase 1 and subsequently, regular KS produced no microscopic or physiologic changes of phase 2.

During phase 1, presumed depletion of Ca^{2+} from the sarcolemma causes its depolarization. Depletion of sarcolemmal Ca^{2+} (and possibly some calcium binding molecules) also leads to irreversible damage to verapamil-sensitive sarcolemmal calcium channels. Unimpeded Ca^{2+} influx from the extracellular space during phase 2 triggers intracellular destructive processes leading to necrosis and sustained contracture of muscle fibers.

CP is a convenient model for the study of sarcolemmal calcium channels and Ca^{2+} induced skeletal muscle cell death.

227.4 ACETYLCHOLINESTERASE MOLECULAR FORMS IN MOUSE DIAPHRAGM. K.A. Skau, Univ. of Cincinnati College of Pharmacy, Cincinnati, OH 45267.

Three major molecular forms of acetylcholinesterase (AChE) are known to exist in rat and mouse diaphragm (HD) muscle. However, some strains of mice have only two major peaks of activity. To further investigate this phenomenon, characteristics of diaphragm AChE were examined in different mouse strains. Two inbred strains of mice and the F1 hybrid generation of these strains were studied. AChE molecular forms were solubilized in a high ionic strength buffer with detergent and separated on 5-20% sucrose density gradients. Enzyme activity was determined by a radiometric assay. ReJ/129 mice exhibited three major forms of AChE activity sedimenting at 4S (G1), 10S (G4) and 16S (A12). These three forms existed in approximately equal proportions. By contrast, C57BL/6J mice lacked a distinct G4 peak. Enzyme activity in the region of the G4 peak was higher than background and probably reflected AChE from intramuscular nerves. In addition, the G1 form was enriched in these animals and was approximately twice the A12 activity. The distribution of AChE in HD from ReJ/129 mice resembled the AChE in fast-twitch extensor digitorum longus muscle of both strains while the AChE from HD of C57BL/6J resembled the enzyme in the slow-twitch soleus. Diaphragms from the F1 hybrids exhibited an intermediate pattern in which a distinct G4 peak was present but was smaller than either the G1 or A12 peaks.

AChE has been shown to be secreted by muscles when cultured for short periods of time after excision. Diaphragms were cultured in oxygenated Hepes buffer and the medium was assayed for AChE molecular forms. Both ReJ/129 and C57BL/6J diaphragms secreted a large G4 and smaller G1 AChE peak. A very small A12 peak was also evident.

The factors regulating the distribution of the AChE molecular forms are not well defined but may involve muscle activity and neuronal trophic factors. The results presented here indicate that different strains of mice exhibit different patterns of AChE in diaphragms and that this distribution is genetically controlled. It remains to be determined whether the diaphragm fiber types or muscle activities differ in these two strains. However, the use of these different strains represents a model system for studying the membrane-bound G4 enzyme characteristics.

227.5 THE HUMAN NEUROMUSCULAR JUNCTION IS STAINED BY MONOCLONAL ANTIBODIES FROM TORPEDO CHOLINERGIC SYNAPTOSOMES. E. Bjornskov*, D.T. Stephenson*, E. Denys*, and P.D. Kushner (SPON: B. Twarog) ALS and Neuromuscular Research Foundation, Pacific Medical Center, San Francisco, CA. 94115

Ten micron thick frozen sections were cut from human intercostal muscle taken from biopsy material. This muscle was chosen because of its high density of innervation. The junctional site was localized by means of rhodamine conjugated to α-bungarotoxin. The monoclonal antibodies were from a library prepared using the Torpedo ray cholinergic synaptosome as immunogen (Kushner, J.Neurochem. 43,1984). The antibody was localized with fluorescein labeled anti-mouse immunoglobulin.

On frozen sections of human intercostal muscle, 21 of the 141 Torpedo monoclonal antibodies (Mab) tested bound to different components of the muscle, nerve and blood vessel. A wide diversity of staining patterns was observed. We have categorized them into five general groups based upon their site of immunolocalization.

Four Mab's bound the myotendenous junction (Tor 77, Tor 81, Tor 140, Tor 177); two Mab's bound the sarcoplasm (Tor 2, Tor 91); one Mab bound precisely the endplate region of the muscle fiber (Tor 244); twelve Mab's bound the peripheral nerve (Tor 23, Tor 25, Tor 58, Tor 75, Tor 103, Tor 132, Tor 145, Tor 190, Tor 201, Tor 219, Tor 232, Tor 233); and six Mab's bound blood vessels (Tor 19, Tor 127, Tor 177, Tor 190, Tor 201, Tor 232).

There was some overlap of staining between the different groups. Three of the Mab's had reactive sites in both the nerve tissue and the blood vessel (Tor 190, Tor 201, Tor 232); one Mab shared antigenic determinants for the myotendenous junction and the blood vessels (Tor 177).

Within each of the five categories, staining variations between the different antibodies were observed. We presume these variations reflect the different molecules comprising each tissue group. Monoclonal antibodies complement traditional cytochemical techniques in examining the cellular and molecular components of the human neuromuscular junction.

227.6 C21A, MOLECULAR FORMS OF ACHE, DENERVATION AND DYSTROPHY OF THE CHICKEN. B.W. Wilson, P.S. Nieberg* and R.K. Entrikin. Avian Sciences, University of California, Davis CA 95616.

Three major molecular forms of AChE are found in chicken fast twitch muscles. Two small (4 and 7 S) globular forms disappear after hatching in normal, are retained in dystrophic and reappear in denervated muscles. A 20S asymmetric form is retained in normal and dystrophic, but lost from denervated muscles. We have shown previously that agents such as corticosteroid 21-acetate (C21A) lower AChE levels in dystrophic chickens. Experiments reported here examine whether C21A affects the patterns of the AChE forms.

Day old dystrophic chicks were injected daily for 5 weeks with 10mg/kg C21A. The biceps muscles were denervated in 4 - 7 week old anesthetized normal chickens, and the birds injected daily for 12-14 days with 10 - 20 mg/kg C21A i.p. After sacrifice, frozen sections of the muscles were examined for size and AChE and detergent extracts were layered onto 5-20 % sucrose gradients and centrifuged at 150,000 xg to separate the AChE forms.

C21A treated dystrophic muscles consistently showed less total AChE than their untreated counterparts, but more than normal controls. AChE forms were similarly affected. For example, relative mean AChE activities (4 birds) of untreated dystrophic muscles were (5-7S): 27; (11S): 4.9; (20S): 11.8 and those for treated dystrophic muscles were (5-7S): 5.7; (11S): 2.0; (20S): 3.9. Denervated normal muscles had higher AChE levels than innervated controls regardless of drug treatment. However, extrajunctional activity and low molecular weight forms of AChE were reduced in denervated muscles from C21A treated birds. 20S AChE forms were not maintained in C21A treated denervated muscle.

The results suggest C21A reduces some effects of denervation and dystrophy on the localization and expression of AChE forms in fast chicken muscle. (Supported in part by the NIH, MDA and NIHR. Dr. Y. Ishikawa, Chiba University, Japan assisted in the denervation experiments.)

227.7 THE RELATIONSHIP OF GLUCOCORTICOID-INDUCED ALTERATION IN ACTIVITY AND MUSCLE WASTING IN THE RAT. R.L. Ruff, Dept. of Physiology Biophysics and Medicine (Neurology), Univ. of Washington, Seattle, WA 98195.

Glucocorticoid treatment can modify the performance of rats in certain behavioral tasks (Micco, D.J., McEwen, B.S. J. Comp. Physiol. Psycho. 94:624-633, 1980). It is thus conceivable that glucocorticoid treatment could suppress normal spontaneous activity and that muscle wasting could be partially due to disuse.

The effect of glucocorticoid treatment on ambulatory and stereotypic (grooming and rearing) activities were recorded in 20 male Sprague-Darley rats (300g) using a digiscan activity monitor (Omicron Instruments). The rats were acclimated to the activity monitors for several days and then baseline activity data was recorded from each animal during four 15 minute periods (two recording periods during the 12 hour dark diurnal cycle and two periods during the 12 hour light cycle) daily for 5 days. Then the animals were randomly divided into two groups of ten animals each. One group received dexamethasone 1.5 mg/kg/day. The second group received an equivalent amount of saline subcutaneously. The animals were then studied for an additional 28 days after treatment started.

Dexamethasone treated animals showed a significant increase in both ambulatory and stereotypic activity in the light period during the initial 14 days of treatment. There was also a slight non-significant increase in dark-associated ambulatory and stereotypic behavior. On this regimen, significant muscle atrophy and reduced force generating capacity are present after 14 days of treatment (Ruff, R.L. et al. Am. J. Physiol. 243:E512-E521, 1982). After 24 days, dexamethasone treated rats showed less dark and light associated activity than control animals.

The data indicates that the initial phase of glucocorticoid-induced muscle atrophy is not associated with reduced activity. However, inactivity may contribute to the muscle atrophy associated with prolonged glucocorticoid treatment. Supported by U.S.P.H.S. grants NS00498 and NS16696.

227.8 EFFECTS OF PERCUTANEOUS HIGH-FREQUENCY ELECTRO-STIMULATION ON THE BREAST MUSCULATURE OF NORMAL AND DYSTROPHIC CHICKENS. M. S. Hudecki, C. M. Pollina*, C. C. Gregorio*, R. R. Heffner and A. T. Caffiero*. Departments of Biological Sciences, Pathology and Physical Therapy, State University of New York at Buffalo, Buffalo, N. Y. 14260.

Percutaneous high-frequency electrical stimulation was applied to each side of the breast musculature of normal line 412 and genetically dystrophic line 413 chickens. Beginning on day 7 ex ovo up to 90 days ex ovo each bird received five stimulations per daily session (3 sessions per week). Approximately 10 mAmps (2500 Hz sine wave modulated at 50 Hz) was supplied to each side of the breast to elicit maximum contraction by the Electrostim 180 (Dual Clinical Model, Micromed Instruments Inc., Montreal, Canada, distributed by Nu-Med, Joliet, IL). Each stimulation cycle was composed of 15 s "on" followed by 50 s "off", and repeated five times. Effects of electro-stimulation on the progression of muscle disease in the dystrophic chicken was monitored by a regularly administered functional test for righting ability, and weekly assays of plasma creatine kinase activities. At the end of each trial the breast musculature (i. e., pectoralis major) was immediately removed from the birds after decapitation for protein, calcium and acetylcholinesterase analyses. Samples were also fixed and immediately processed for morphometric and histochemical quantitations. These include: fiber necrosis, regeneration, vacuolation, inflammation, and diameters, and metabolic fiber typing. Further on-going study involves electro-stimulation trials in combination with the intraperitoneal administration of the branched-chain amino acids or proteinase inhibitors.

This study was supported by grants from the Muscular Dystrophy Association, National Institute of Neurological and Communicative Disorders and Stroke, and Biomedical Research Support Grant to the State University of New York at Buffalo from the U. S. Public Health Service. The authors thank Mr. William De Vries (Pres., Nu-Med Surgical Corp.) for loan of the Electrostim 180.

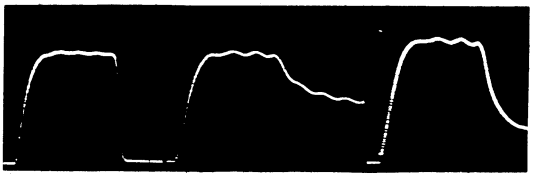
227.9 BIOMECHANICAL BASIS FOR THE INCREASED STIFFNESS OF AVIAN DYSTROPHIC MUSCLE. H. Feit and M. Kawai*, Departments of Neurology and Cell Biology, Univ. Tx. Hlth. Sci. Ctr., Dallas, Tx 75235 and Department of Anatomy and Cell Biology, Columbia University, N.Y. 10032

Resting tension and stiffness as a function of muscle length were measured on isolated small bundles of chemically skinned myofibers obtained from normal and dystrophic chicken pectoral muscles. In dystrophic muscle, tension rose steeply and stiffness was much higher than normal. This stiffness was not removed by extraction with either 0.6 M KI or with 5 M guanidine HCl mixed with 1% mercaptoethanol, implying that a large, covalently cross-linked protein(s) underlies the excessive stiffness of dystrophic muscle. Exposure to collagenase did not affect the results. Additional experiments on normal tendon showed that the collagenase digestion conditions were adequate. Nyquist plots (frequency response functions plotted in elastic and viscous axes) obtained from normal and dystrophic myofibers during Ca-activated isometric contraction were very similar and contained three exponential processes characteristic of fast-twitch muscle. The unaltered appearance of the Nyquist plot of dystrophic muscle implies that cross-bridge function is normal and the stiffness of dystrophic muscle is not a consequence of abnormal or rigor cross-bridge formation. Our studies also indicate that the characteristic slowing of force development and relaxation in dystrophic muscle may be a consequence of the increased resting stiffness and does not involve a fast-to-slow transformation of muscle types. Biochemical evidence suggests that the increased stiffness of dystrophic muscle depends on an abnormal form of collagen which is collagenase-insensitive by virtue of excessive cross-linking. This abnormal form of collagen has been named "dystrophin" because it is present in dystrophic muscle and absent in normal muscle. Simple histological procedures for demonstrating dystrophin have been developed and will be applied to human forms of muscular dystrophy. Our work supports the concept that muscular dystrophy represents an abnormal form of fibrosis resulting in a mismatch of compliance between the extracellular matrix and the myoplasm.

227.10 NEUROMUSCULAR DISORDERS: MYOTONIA AND MUSCULAR DYSTROPHY IN MICE. R.K. Entrikin, R.T. Abresch*, D.B. Larson*, N.A. Levine*, R.B. Sharman*. Physical Medicine & Rehabilitation, University of California, Davis, CA 95616.

A recessive neuromuscular disorder in mice, "myotonia," was first-described by Heller et al., (J. Neuroscience 7: 924-933, 1982). We have established a colony and begun studies to further characterize and determine the basis of skeletal muscle dysfunction in affected homozygotes (mto/mto). The animals seem normal at rest, except for smaller body size than unaffected littermates. When walking the forelimbs move in a normal manner, but the hindlimbs are extended, resulting in a stiff-legged, "waddling" hindlimb gait. Unlike true myotonia there is no apparent decrease in "stiffness" during repeated movement. EMG insertional activity consists of bizarre high-frequency discharges that last several seconds after cessation of electrode movement in anesthetized mice. Although reminiscent of myotonic EMG activity, the murine pattern differs by the relative lack of typical "dive-bomber" discharges. In vitro contractile studies were performed on EDL muscles of 4 mto/mto, 4 +/? (unaffected) littermates, and 3 "dystrophic" (129B6F) mice killed by cervical disarticulation at 5-8 weeks of age. Dystrophic muscles had abnormalities only in tension parameters, but mto/mto muscles had prominent, consistent abnormalities in time-parameters of contraction. Following tetanic stimulation (67, 1-ms pulses) +/? muscles rapidly returned to baseline tension levels (Fig A), but mto/mto muscles had greatly prolonged relaxation times (Fig B). Interestingly, the delayed relaxation in vitro was reversibly antagonized by 1.5 x 10^{-2} mg/ml tubocurarine in the bath solution (Fig C). From these studies it is not clear whether the "myotonic" mouse is a form of "true" myotonia. (Supported by NIHR Grant G008300078).

A B C



227.11 COMPUTERIZED QUANTITATION OF EMG ACTIVITY IN THE STUDY OF FENTANYL-INDUCED MUSCLE RIGIDITY IN RATS. G. Polansky* and P.D. Thut. Dept of Pharmacology, Univ. of Maryland, Sch. of Dentistry, Baltimore, MD 21201.

It has long been observed that morphine and other opioids can produce distinct muscular rigidity. Most studies of the opioid-induced rigidity are based on relatively subjective tests (i.e. ranking scale from 1 to 5 of observed rigidity). The measurement of muscular rigidity by electromyography (EMG) and the quantitation of the EMG by integration and computer digitization and storage offers many advantages over previous methods.

For EMG recordings, stainless steel wires are sutured approximately 7 to 10 mm apart in the left spinal trapezius muscle of a rat. Three stainless steel screws are implanted in the skull. One of the screws is soldered to a wire and acts as a ground. The other two screws serve as anchors for a head piece to which are attached the electrodes and ground. The head piece is secured to the skull with dental acrylic. EMG activity is processed by a Gould Universal Amplifier and a Gould Integrator. Output from the integrator is appropriately amplified and then fed to a Mountain Hardware analog to digital converter which is resident in a peripheral slot of an Apple II Plus microcomputer. A Thunderware clock, installed in another slot, is used by the Apple for timing of computer controlled resets of the integrator. Immediately preceding each integrator reset, the peak integrated value is digitized and stored in memory. At the end of the experiment, all data are stored on a 5-1/4" floppy disk. The computer is used to perform the necessary calculations, which greatly expedites data analysis. All drugs are administered through an indwelling jugular cannula.

The opioid fentanyl was used to produce rigidity and to test this method. Percent change in the integrated EMG was measured over a dose range of 10 to 79 μg/kg. A linear log dose-response was observed.

This method of quantitation of muscular rigidity is less subjective and therefore helps eliminate experimental bias and allows better comparisons of results between laboratories. It also may be a more sensitive measure of the degree of rigidity than other methods.

231 SYMPOSIUM: DEVELOPMENT OF CNS FUNCTION IN UTERO. C.J. Shatz, Stanford Univ. Sch. of Med. and G.M. Shepherd, Yale Univ. Sch. of Med., (chairpersons); J.C. Birnholz*, Rush Med. College; C.M. Mistretta, Univ. of Michigan; P.E. Pedersen, Yale Univ. Sch. of Med.

New evidence suggests that pathways within the mammalian central nervous system (CNS) function or are capable of function long before birth. This symposium considers four examples of function in utero. The first is the development of taste function (C.M. Mistretta). Gustatory receptors appear early in fetal sheep, and since the fetus periodically swallows large volumes of amniotic fluid, potential stimuli are available. In vivo microelectrode recordings indicate that peripheral taste nerves and the second order taste cells are functional throughout the last half of gestation. However, although responses to specific taste stimuli are present, the system does not respond in a mature manner at the onset of function. Olfactory function also appears to begin in utero; 14C-2-deoxy-D-glucose injection into the pregnant rat reveals differentially high uptake in the fetal accessory olfactory bulbs (P.E. Pedersen and G.M. Shepherd). In addition, injection of odorous substances into the amniotic fluid influences the odor cue to which newborns suckle, demonstrating that the intrauterine environment interacts with developing olfactory function. Studies of the cat's visual system (C.J. Shatz) show that functional connections are present between retinal ganglion cell axons and their target thalamic neurons throughout the last half of gestation. Microelectrode recordings made from the fetal thalamus in vitro show that the pattern of synaptic activity changes progressively as ganglion cell axons from the two eyes sort out into their adult layers, suggesting that function itself may play a role in producing the final adult pattern of connectivity. Studies of human fetal development by means of ultrasound (J.C. Birnholz) show that a wide range of independent motor patterns is present by 20 weeks of gestation. Spontaneous eye movements, and blink-startle responses to sound and light, can be detected indicating that the human fetus can respond to external stimuli. These observations demonstrate that a considerable degree of CNS functional development occurs in utero, and therefore that some aspects of the mammalian CNS are more mature at birth than heretofore supposed. (Thanks to Acuson for a travel grant for J.C.B.)

232 SYMPOSIUM. MECHANISMS OF TRANSMITTER RELEASE. J.P. Tremblay, Univ. Laval (Chairperson); C. Erxleben and M.E. Kriebel, State Univ. of N.Y.; S. Yazulla, SUNY; Y. Dunant, Medical School, Geneve, Suisse; J. Vautrin and J. Mambrini, Univ. Paris XII, France; M.M. Poo, Univ. Cal. Irvine.

The symposium will present different experimental results indicating that the neurotransmitter release may be quantal, subquantal or non quantal. Some of these results may be interpreted within the vesicular theory, others suggest non vesicular mechanisms of release. Drs Erxleben and M.E. Kriebel will present the amplitude distributions and time characteristics of miniature endplate currents (MEPCs). At -140 mV holding-potential (30°C; 25mM Ca-saline) MEPC amplitude histograms showed integral multiple peaks with intervals of 0.44 ± 0.035 nA. Peak intervals, but not their number, changed with temperature holding potential and after ACh-esterase block, demonstrating that the quantum of transmitter is composed of 11 subunits. Dr Yazulla studies the ^{3}H-GABA efflux from horizontal cells evoked in superfused isolated retinas by acidic amino acids, potassium and darkness. Evoked ^{3}H-GABA efflux was voltage and sodium dependent, calcium independent and inhibited by nipecotic acid and dopamine. His results suggest a non-conventional mode of transmitter GABA release by goldfish horizontal cells. Dr Dunant reports that in the Torpedo electric organ, transmission of a single nerve impulse is accompanied by a brief increase in the number of intramembrane particles in the presynaptic membrane. At the same time, there is rapid utilization and renewal of the extravesicular pool of acetylcholine, whereas vesicular acetylcholine remains unchanged. He concludes from these and other results that acetylcholine release is ensured by a membrane-bound operating mechanism which uses cytoplasmic acetylcholine. On the other hand, regarding acetylcholine, calcium and protein metabolism, synaptic vesicles play several key roles which will be presented. Drs Vautrin and Mambrini investigate the frog unitary evoked potential (UEP) in low calcium solution. They report stepwise variations of not only in the UEP but also in the latency between the stimulation and the onset of UEP. This clustering of latencies suggests that the releases arise from a limited number of active sites at more or less regular intervals (10-20 um) along the nerve terminal. Dr Poo studies the release of acetylcholine (ACh) from embryonic Xenopus neurons during the early phase of synaptogenesis by intracellular and patch-clamp recording techniques. Three types of release will be discussed: the ACh release from the nerve growth cone prior to the contact with the muscle cell, the quantal release of ACh after nerve-muscle contact, and the steady "non-quantal" release during the early phase of synapse maturation.

233.1 **MONOCLONAL ANTIBODIES WITH DEVELOPMENTALLY VARYING BINDING DISTRIBUTIONS IN THE RAT NERVOUS SYSTEM.** M.Constantine-Paton & C. J. Barnstable. The Rockefeller University, New York, NY 10021.

Complete understanding of CNS development will require probes for biochemical changes in the intact tissue that may not be accompanied by obvious alterations in structure. To obtain such probes we have begun to screen monoclonal antibodies raised against fetal rat retina (E12-20). We report here on three antibodies whose binding distributions in neural tissue show dramatic increases and then decreases with age.

The antibody INDIANA (INDI) binds to all cells comprising the embryonic and extraembryonic epithelia of the early embryo (E6-7). By E9 INDI binding is localized to the peripheral ganglia and all neuroepithelia. This distribution is retained throughout fetal life even though many cells in these regions begin differentiation during this period. In the mature CNS INDI binding is much more restricted. In retina, for example, photoreceptor inner segments are the predominant binding site.

A second antibody, JONES, exhibits a punctate membrane associated pattern of labelling in the region of the ventral forebrain and the eye evagination around E9. In the CNS of the 12-13 day embryo JONES may indicate early subdivisions of mature brain regions. Its punctate staining appears in patches sometimes showing abrupt discontinuities on morphologically continuous sheets of cells. A similar pattern of JONES binding is observed on the cell membranes of clumps of cells within a cloned somatic cell hybrid line made from the fusion of a mouse neuroblastoma with rat embryonic cortical cells. JONES does not bind to the E12 retina but by PN1 binding is heavy and may be graded along all retinal layers. In the mature retina JONES binds only in the outer plexiform layer.

A third antibody, ADAMS, does not label the early embryo or the retina until late gestation. However in the late fetus and the neonate the antigen is distributed throughout the retinal epithelium. In the adult ADAMS is selective for Muller cells.

It is clear that monoclonal antibodies antibodies can be used to detect developmental changes that are not obvious by other anatomical or biochemical criteria. Still to be investigated is the question of whether the distribution of antibody binding is truly predictive of any subsequent developmental changes in the labeled cells.

233.2 NEURONAL DEVELOPMENT IN THE DROSOPHILA RETINA: FROM MONOCLONAL ANTIBODY TO GENE. T. R. Venkatesh, S. L. Zipursky, D. B. Teplow and S. Benzer. Div. of Biol., Caltech, Pasadena, CA 91125.

The reiterative pattern of photoreceptor cells in the compound eye of Drosophila is established in the eye imaginal disc during the third larval instar. The molecular genetic mechanisms that give rise to this precise pattern are unknown. To approach this issue we have used monoclonal antibodies (MAbs) to characterize molecules associated with specific patterns and to isolate the genes encoding these molecules (Zipursky et al., Cell 36, 15-26, 1984).

MAb 24B10 is specific to photoreceptor cells and their axons; the staining appears early during photoreceptor development. The polypeptide recognized by MAb 24B10 (Ag24B10) has been purified and a partial amino acid sequence at its N-terminus has been determined. An oligonucleotide probe corresponding to a portion of this sequence was synthesized and used to screen clones from a Drosophila genomic library in phage λ. A clone was isolated which hybridized with the probe. On Southern blots made with restriction digests of genomic DNA, or the cloned DNA, identical fragments were lit up by the probe. The genomic DNA clone from the library contained a 13.5 kb insert. This was cut with enzymes EcoR1 and Pst1 to generate a 600 base pair fragment, which hybridized to the oligonucleotide probe. This fragment was subcloned into plasmid puc8 to generate large amounts of the DNA. A 250 base pair fragment that would label only at the Hinf end was generated by cutting the EcoR1, Pst1 fragment with a series of restriction enzymes. The 250 base pair fragment was sequenced by the Maxam and Gilbert method. The DNA sequence showed an open reading frame containing the sequence corresponding to the known N-terminal amino acid sequence of Ag24B10.

233.3 ABERRANT PATTERN FORMATION OF THE DEVELOPING PHOTORECEPTOR ARRAY IN DROSOPHILA MUTANTS. P.J. Renfranz* and S. Benzer. Div. of Biol., Caltech, Pasadena, CA 91125.

The eye-antennal imaginal disc of the 3rd instar Drosophila larva is a unique system for studying developmental pattern formation. A morphogenetic furrow sweeps anteriorly across the eye disc, leaving behind a precise array of clusters of photoreceptor cells which send axons to the optic lobe. Lebovitz and Ready (Neurosci. Abstr. 8, 702, 1982) showed an antigenic defect in eye discs of the mutant glass. We have used a panel of five monoclonal antibodies (MAbs) which highlight specific pattern elements to analyze various mutant discs. MAb 3E1 lights up the region ahead of the furrow, MAb 22G8 the furrow itself, MAb 6D6 a network of cells surrounding each cluster, MAb 22C10 axons in general, and MAb 24B10 photoreceptor cells and their axons (Zipursky et al., Cell 36, 15-26, 1984).

Mutants such as split, rough and roughex, in which adult eyes are of normal size but irregular in facet arrangement, showed no major defects in the disc; the five antibody staining patterns were similar to those in wild type.

In the double mutant Star, Enhancer of Star, the adult eye is reduced to a small, central faceted region, and the optic lobe is small and disarrayed. Development in the disc appeared normal in early stages: the patterns of MAbs 3E1 and 22G8 were comparable to wild type and, immediately behind the furrow, a field of clusters was visible with MAbs 22C10 and 24B10. However, older clusters in the posterior disc had lost their normal reactivity with the latter two MAbs. This developmental defect is thus manifest at the disc stage.

Drop mutant adult eyes are reduced to only a few facets in the posterior region of the eye; in the disc, extensive cell death occurs ahead of and behind the furrow. MAbs 3E1 and 22G8 showed that the furrow itself was already distorted. MAbs 6D6, 22C10 and 24B10 revealed a severely reduced number of clusters; only one or two columns of clusters matured at the posterior end of the disc. The eye of the mutant Irregular facets is devoid of most facets. In the disc, the furrow was irregular and the few clusters were abnormally oriented to the disc surface. Thus, these mutations affect eye development at an early imaginal disc stage.

Further characterization of these genetic abnormalities in development may be useful in dissecting the complex events associated with pattern formation.

233.4 INITIAL EVENTS OF LAMINATION IN THE MAMMALIAN RETINA. Colin J. Barnstable & Martha Constantine-Paton. The Rockefeller University, New York, NY 10021.

The mechanism(s) by which the laminated structure of the mammalian retina is set up is not known. The presumed stem cell pool from which retinal cell types arise consists of ventricular cells that extend processes across the tissue to both retinal surfaces. After their final mitoses, cells move to their correct positions and acquire adult characteristics. To examine the development of retinal cell types and mechanisms of lamination we have used well characterised cell-type specific monoclonal antibodies to label the developing retina.

Antibody HPC-1 labels cell bodies and processes of amacrine cells in adult retina. In late embryonic and early postnatal tissue HPC-1 positive cell bodies can be found in the forming amacrine and ganglion cell layers. The developing inner plexiform layer is also labelled. In addition HPC-1 labelled radial cells were also found in the outer portions of the retina and some of the radial processes could be followed to the ventricular surface. This suggests that amacrine cells show distinct differentiation and migration soon after their final mitosis and argues against recruitment into the amacrine cell layer as a result of the radial position in which undifferentiated cells settle.

Early photoreceptor development has been monitored with antibody RET-P1. Although a few labelled cells were found in both central and peripheral retina at birth, by PN 3 there was a clear central to peripheral gradient of labelling. Although the photoreceptor layer was not as defined as in the adult most labelled cells were confined to the outer third of the retina.

Using both histological staining of lum sections of methacrylate embedded retinas and immunocytochemical labelling of cryostat sections, we have found that differentiated horizontal cells are present at birth. These cells still have radial processes connecting them to the ventricular surface when they begin to elaborate tangential processes in the otherwise undifferentiated outer plexiform layer. It is not yet clear whether these radial processes are used by the cells to position their cell bodies correctly. However the early appearance of their lateral processes suggests that these cells may play an important role in the structuring of cell and synaptic layers in the outer retina.

233.5 **CELL INTERACTIONS DURING EARLY DEVELOPMENT OF THE CNS IN THE GRASSHOPPER EMBRYO.** Chris Q. Doe*, and Corey S. Goodman (SPON: A. Harris). Dept. of Biol. Sci., Stanford Univ., Stanford, CA 94305.

Development of the grasshopper CNS begins following gastrulation, when the embryo consists of a thin ventral strip of ectoderm and dorsolateral mesoderm. During the next 5% of embryonic development ectodermal cells delaminate, losing their filopodia and their contact with the ventral surface. It is from groups of these cuboidal delaminated cells (DCs) that each neuroblast (NB) forms. Unlike ectodermal cells or DCs, NBs form in a precise temporal and spatial pattern. Each segment has a characteristic number of NBs, and every NB can be individually identified according to its time of formation, position in the neuroepithelium, and the stereotyped family of neurons produced. We are interested in how the seemingly uniform ectoderm produces this array of unique NBs.

Neither DCs nor NBs form simultaneously; rather, early arising NBs are seperated by groups of DCs that will ultimately produce the remaining NBs. Using laser ablations of cells in embryos cultured in vitro we showed that any one of the DCs between NB 7-2 and 7-4 could produce NB 7-3 (Taghert, Doe, Goodman, Nature, 1984). By doing in ovo kills with the laser microbeam, we discovered that the DCs in position 7-3 could also replace NB 7-2 or 7-4 after their ablation. This result has been confirmed on 5 other NBs (n 20 for each). Regulation of a mature NB only occurs when adjacent DCs exist, there is no correlation with age of the killed NB or segment age.

Several results suggest that the fate of the regulated NB is positionally determined: (1) when NB 7-4 is killed and regulation occurs, there is never duplication of NB 7-3 progeny, which might be expected if the 7-3-position DCs were already determined and yet produced two NBs (at the 7-3 and 7-4 positions); (2) when NB 1-1 is killed, and regulation occurs (presumably from a cell in the DC group at the 1-2 postion), individual neurons normally produced by NB 1-1 are observed. Apparently DCs from adjacent postions in the neuroepithelium assume the NB 1-1 fate.

We conclude from these results that the pre-neuronal cell type, DCs, are not determined, but can assume two or more NB fates, and that specification of fate amoung ectodermal cells and DCs is positionally, not lineally, regulated.

233.6 **CELL INTERACTIONS UNDERLYING THE ACQUISITION OF SPECIFIC FATES BY SIBLING NEURONS DURING GRASSHOPPER EMBRYOGENESIS.** John Y. Kuwada and Corey S. Goodman. Dept. of Biol. Sci., Stanford University, Stanford, CA 94305.

In insect embryos, neurons are produced by ganglion mother cells (GMCs) which arise from asymmetric divisions of neuroblasts (NBs), and by midline precursors (MPs). In both cases, pairs of sibling neurons are generated by symmetric divisions of either MPs or GMCs; such pairs of sibling neuronal progeny are a common feature of insect neurogenesis. In grasshopper embryos, the accessibility of the neuronal precursor cells and their progeny throughout development makes it possible to undertake a cellular analysis of neuronal determination.

We previously reported on studies of the sibling progeny of MP3 which develop into the morphologically distinct H cell and H cell sib. If one of the two cells was ablated within 5 hrs of their birth, the remaining cell developed into the H cell sib. When one of the cells was ablated during the next 5 hrs (and still before axonogenesis), 50% of the progeny developed into the H cell and 50% into the H cell sib. These results indicated that the MP3 progeny are initially equivalent at birth. At this stage there is a hierarchy of fates with the H sib being dominant. Furthermore, a few hours later in development, the two cells acquired their unique determination by cell interactions (Kuwada and Goodman, Neurosci. Abstr. 1983).

We have now extended this analysis of neuronal determination to a pair of sibling neuronal progeny which arise from the first GMC of NB 1-1: the morphologically distinct aCC and pCC neurons. As with the MP3 progeny, ablation of one of the first pair of NB 1-1 progeny within 5 hrs of their birth results in fate regulation with the remaining cell developing into the pCC. When ablations are made between 5 and 10 hrs after their birth, the remaining cell becomes either the aCC or pCC with equal probability. These combined results suggest that sibling neuronal progeny arising from both NBs and MPs are 1) initially equivalent, 2) become uniquely determined by early cell interactions, and 3) exhibit a hierarchy of fates. Pairs of sibling neurons are ubiquitous in the insect CNS, and we propose that such pairs which ultimately have unique fates are born initially equivalent with a hierarchy of cell fates. Moreover, their cell-specific determination involves cell interactions which occur early in their development.
(Supported by NIH grants NS20299 to JYK and NS18366 to CSG.)

233.7 POSITIONAL CONTROL OF MITOTIC WITHDRAWAL IN THE DEVELOPING EYE OF XENOPUS: AN ANALYSIS OF GENETIC CHIMERAE. R.K. Hunt* and J.S. Cohen* (SPON: W.M. Cowan). The Salk Institute, La Jolla, CA 92037.

In Xenopus, the back of the embryonic eye (BOE) becomes mitotically quiescent at stages 28-38 (2.5-4 days post-fertilization). Thereafter, mitosis is confined to a germinal ring on the front of the early larval eye: the ring is 'bilayered', with an outer melanogenic layer that contributes post-mitotic cells to the pigment retinal epithelium (PRE) proximally and to the iris distally, and an inner neurogenic layer which adds post-mitotic neurons to the rim of the neural retina. The 'melanogenic' layer (iris, germinal ring and PRE) offers a useful model for experimental studies on the control of mitotic withdrawal, because chimerae prepared by grafting small groups of eye cells from pigmented to albino embryos can be studied by serial photography of the evolving black/white pattern in the living eye. When a small patch of germinal ring/presumptive iris is transposed orthotopically, from a stage 36-38 pigmented donor eye (wild-type or hybrids of X. laevis and X. borealis) into a stage 32-36 albino host eye, the patch heals successfully into the host germinal ring in about 70% of cases and, thereafter, begins to add cells to the distal rim of the PRE —forming an ever-elongating black sector in the otherwise albino eye — and (if the implant, when healed, abuts on the pupillary margin) contributes a black sector-territory to the iris as well (N=42). By contrast, when small patches of PRE from the mitotically quiescent BOE are orthotopically transposed, the tiny black patch stops growing within two days. Thereafter, the patch remains constant in size and (as additional annuli of 'all albino' cells are added to the rim of the PRE) is progressively displaced from the eye rim with subsequent larval growth (N=15). Heterotopic grafts, from the germinal ring/presumptive iris into the PRE in the mitotically quiescent BOE also cease growth within two days and behave thereafter in a manner indistinguishable from orthotopic BOE grafts (N=16). Sectioned material at mid-larval stages shows a black, but cytologically unremarkable patch of PRE. By contrast, PRE grafts from mitotically quiescent BOE into the germinal ring/iris typically recommence growth (33/45 cases) and either form iris (N=7) or an elongating black sector in both PRE and iris (N=26) in a manner similar to orthotopic germinal ring grafts. Sectioned material of these latter cases shows fully differentiated graft-derived iris and PRE, indistinguishable in the light microscope from the PRE-iris sectors formed by orthotopic grafts of germinal ring/iris. We conclude that positional cues, perhaps from the lens rudiment, participate in the control of mitosis in the developing eye. (Supported by NSF).

233.8 A MONOCLONAL ANTIBODY REVEALS EARLY DIFFERENCES BETWEEN NEURAL AND NON-NEURAL ECTODERM IN XENOPUS LAEVIS EMBRYOS. R. M. Akers, C. R. Phillips* and N. K. Wessells*. Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305 and Dept. of Zoology, Univ. of Calif., Berkeley, CA 94720

The events involved in the earliest stages of nervous system development are poorly understood. The appearance of the neural plate and its subsequent segregation from the surrounding ectoderm may involve alterations in intercellular adhesion and/or recognition. Monoclonal antibodies provide one means by which such developmental events may be studied. Toward this end, we have generated monoclonal antibodies to membranes isolated from Xenopus laevis embryos at the onset of neural tube closure (Nieuwkoop and Faber st. 19). Hybridoma supernatants were screened on frozen sections, and several antibodies recognizing subsets of embryonic cells were detected. One antibody, ECTO 1, binds to cells of the non-neural ectoderm, but not to cells of the developing neural plate. The ECTO 1 antigen is not detectable in embryos before or during gastrulation; it appears in the non-neural ectoderm at the onset of neural plate formation (st. 13) and is maintained throughout early tailbud stages (the latest stages examined). Specialized regions of ectoderm arising later in development (otic placode, lens, and cement gland) also lack this antigen. Western immunoblot analysis shows the antigen to be a single band with an apparent molecular weight on SDS gels of 300-400,000 Daltons. Although the antigen is known to be membrane-associated, its specific subcellular localization is not known.

Specialized ectodermal structures are thought to arise via inductive interactions with surrounding tissues. Such interactions may thus control the expression of the ECTO 1 antigen. In order to examine this possibility, we isolated explants of ectoderm from embryos during gastrulation, at which point it is possible to identify presumptive neural and non-neural tissues. After 12 hours in culture, explants of both neural and non-neural ectoderm were found to express the ECTO 1 antigen. Thus, contact between presumptive neural ectoderm and surrounding tissues in vivo may inhibit the expression of the ECTO 1 antigen. This antigen may prove to be a valuable marker for future studies of inductive interactions involving ectodermal tissues.

[Supported by grants from the N. I. H. (HD 04708) and N. S. F. (PCM 83-092621)].

233.9 STEPWISE COMMITMENT OF BLAST CELLS TO ONE OF TWO ALTERNATIVE DEVELOPMENTAL PATHWAYS IN THE EMBRYO OF THE LEECH. M. Shankland and D.A. Weisblat. Depts. of Molecular Biology and Zoology, U. of California, Berkeley, CA 94720.

The ectoderm of the leech embryo contains two parallel columns of blast cells--the o and p bandlets--which during normal development give rise to distinct O and P patterns of both neural and epidermal descendants. Previous work has shown that the o blast cells are initially capable of following either the O or P developmental pathway, but are somehow diverted into the O pathway from the P pathway by the presence of the other cell line. If the P cell line is eliminated by ablating its ancestral blastomere, then the o blast cells are not diverted and follow the P pathway instead.

To examine this interaction in more detail we selectively photoablated the p bandlet and followed the development of o blast cells located adjacent to the lesion. Photoablation was accomplished by labelling the p blast cells with a fluorescent lineage tracer injected into their ancestral blastomere, and then exposing this tracer to intense photo-excitation within the living cell. The developmental fate of the o blast cells was determined by labelling them with HRP injected into their ancestral blastomere, and staining their descendant tissues at a later stage of embryogenesis. Photo-ablations performed soon after the o and p blast cells come into contact can prevent the normal diversion of the o blast cells into the O pathway. However, later photoablations do not have this effect, suggesting that the o blast cells have lost their dependence upon interaction with the p blast cells and become--within the context of these experiments--autonomously committed to the O pathway.

Each o blast cell becomes committed to the O pathway in a sequence of three discrete events separated by intervals of approximately 5 hr. Each event renders a different subset of the blast cell's descendant clone unresponsive to any subsequent p bandlet ablation. These three commitment events occur in phase with and just prior to the first three blast cell divisions, and may reflect the sequential commitment of different blast cell daughters.

233.10 **DEVELOPMENT OF THE MAMMALIAN CNS EXAMINED WITH MOLECULAR PROBES,** R. McKay [1], O. Sundin [2], S. Hockfield [3]. 1. Biology & Whittaker College, MIT, Boston, MA; 2. MRC Mol Biol Lab, Cambridge, England; 3. Cold Spring Harbor, NY

We have used hybridoma and recombinant DNA technologies to generate probes for molecular differences present during the development of the mammalian central nervous system.

Using monoclonal antibodies generated against the adult CNS we have shown that many antigens present in mature neurons are expressed late in development, long after the terminal mitosis and the expression of much of the adult phenotype. We have generated another group of monoclonal antibodies against the embryonic CNS and have used these to identify major cell types and to study the developmental sequence of antigen expression in the embryonic nervous system. These studies have shown that the number of molecular differences among both developing and adult neurons is likely to be great. They have also shown that the expression of many antigens is under developmental control and that there may be a large number of antigens that are expressd only transiently during neurogenesis.

The large number of potential antigens of interest and the difficulty of readily obtaining suitable probes using hybridoma technology suggested that another strategy might provide probes with higher efficiency. We have now made a cDNA library from mRNA purified from the embryonic CNS in the Trp expression vector. Plus-minus screening of this library using probes from embryonic liver and CNS has shown that many bacterial colonies contain DNA inserts that are more prevalent in CNS than in liver A+ mRNA. In order to look for selective mRNA expression at the single cell level we are using in situ hybridization to sections of E15 embryos. These studies have shown that there are a number of molecular differences in the embryonic CNS, both in the spatial and temporal patterns of expression of proteins and mRNA's.

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233.11 PHOTOSENSITIZATION AND KILLING OF NEURONAL PRECURSOR CELLS BY MEANS OF BUDR AND NEAR-UV IRRADIATION. Kenneth C. Smith*, Murray S. Flaster, and Eduardo R. Macagno. Dept. of Biological Sciences, Columbia University, New York, NY 10027

We have used the UV photosensitizing effects of the thymidine analogue 5'-bromodeoxyuridine (BUDR) to selectively kill cells undergoing mitosis at a particular time in the development of the nervous system of Daphnia magna. Embryos were injected at 41 hours of development with ^{3}H-BUDR to give a concentration in the embryo of 3uM. One hour later, the left side of the eye-lamina anlage was irradiated for 10, 20, or 100 sec with a focused UV microbeam whose wavelength peak was 310nm. No effects were seen when the irradiation times were 10 or 20 sec, but irradiation for 100 sec caused the loss of an average of 16 out of the 88 photoreceptors normally present per side of the eye. There was no cell loss on the unirradiated right side of the eye. Control 100 sec irradiations, without BUDR, seldom caused deletion of photoreceptors. Autoradiography of specimens serially sectioned at one micron confirms that the ^{3}H-BUDR is being incorporated by mitotic cells in both the eye and the optic lamina. Light microscopic examination of the serial sections fails to reveal any major structural effects of the BUDR injection. We are presently refining this technique for use in the selective killing of the target cells of the photoreceptors in the optic lamina in order to test the role of cell-cell interactions in the differentiation of these neurons. (Supported in part by NIH Grant NS-14946).

233.12 MUTATIONS AFFECTING CELLS OF THE TOUCH REFLEX CIRCUIT IN CAENORHABDITIS ELEGANS. M. Chalfie, Dept. Biol. Sci., Columbia University, New York, NY 10027.

The nematode Caenorhabditis elegans moves forward or backward depending on whether the animal is touched on the tail or head, respectively. These touch reflexes involve two different circuits each with touch receptors, interneurons, and motor neurons. Removal of each class of neuron by laser ablation results in animals with distinctive and characteristic behavioral defects. Mutants showing similar defects are also seen. Over 250 touch insensitive mutants have been isolated. Although most mutants are touch insensitive in the head and tail, some animals show a differential touch defect. Most of the mutations producing differential effects are in genes for which other alleles exist that produce completely insensitive animals. For example, some of the recessive mutations in mec-12 result in animals that are head insensitive; others produce animals that are insensitive in the head and tail (three dominant mutations in this gene produce head insensitive animals in heterozygotes but completely insensitive animals in homozygotes). These different phenotypes are probably not the result of differential gene activity. The weaker mec-12 mutants fail to form a terminal branch; the stonger ones in addition have less of the microtubules that are known to be required for sensory transduction. Since the synapses made on the branch are important for anterior touch only, the differential defect seen in these animals is likely to be a consequence of the geometry of the neuronal circuitry.

Two mutants with tail insensitivity have been studied. One mutation (u38) results in the death of the interneurons onto which the tail touch cells synapse. The other (u282) alters cell lineages so that the tail touch cells (but not the anterior touch cells) are not produced.

These data suggest that although particular mutations can produce differentially touch-insensitive animals, few, if any, genes appear to be important in determining the differences in the synaptic connections made by the C. elegans touch cells. It is thus possible that the touch cells are not intrinsically different from one another, but form particular synapses as the result of extrinsic factors.

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233.13 MICROFILAMENT ORGANIZATION IN REACTIVE ASTROCYTES IN CULTURE. S. Fedoroff, I. Ahmed‡ M. Opas*and V.I. Kalnins* Departments of Anatomy, University of Saskatchewan, Saskatoon, Sask. S7N OWO, and University of Toronto, Toronto, Ontario, M5S 1A8, Canada

Astrocyte precursor cells isolated from brain can grow and differentiate in culture. If dBcAMP is added to cultures after they have reached the astroblast stage of differentiation (vimentin$^+$, GFAP$^+$) the cells transform into large stellate cells with increased amount of GFAP, increased numbers of mitochondria and increased rate of cell respiration. These cells are considerably larger than the normal fibrous astrocytes in situ and in vitro. According to morphology, immunocytochemistry and morphometric measurements, these cells are similar to reactive astrocytes which form in situ in response to injury. We have previously reported the organization of microfilaments (MF) in cells of the astrocyte cell lineage. Now we have extended these studies to reactive astrocytes in culture by examining their microfilament organization, using NBD-phallacidine. In astroblasts the perinuclear region has relatively few MF bundles; however, toward the periphery of the cell many fine interlacing bundles of MF are found. These tend to be concentrically arranged and loosely organized. Some bundles of MF extend into the cell processes and insert into the cell membrane along the intercellular junctions. In reactive astrocytes which form in cultures from astroblasts, the loosely arranged circumferentially oriented bundles of MF found in astroblasts, are condensed into a circular, strongly staining ring and only some smaller bundles of MF extend into the cell process. It is of interest that normal fibrous astrocytes, in contrast to astroblasts and reactive astrocytes, have very little polymerized actin. The astroblasts are fairly motile in cultures and have adhesion plaques along the substratum but neither reactive astrocytes nor fibrous astrocytes have adhesion plaques and they are relatively non-motile. Supported by MRC Canada Grants MT-4325 to SF and MT-3302 to VIK.

BLOOD BRAIN BARRIER I

234.1 POLARITY OF BLOOD-BRAIN BARRIER VALPROATE UPTAKE. E. M. Cornford, C. P. Diep* and W. M. Pardridge. Epilepsy Center, V.A. Wadsworth Med Ctr, and UCLA School of Medicine, Los Angeles, CA 90024.

The intracarotid single-injection technique of Oldendorf has been extensively used to study blood-brain barrier (BBB) functions in vivo. Brain uptake indices (= BUIs) provide an estimate of the extraction of the test compound relative to the extraction of a reference compound (such as tritiated water). For example, in the presence of 0.1% albumin, the valproate BUI = 42%. This type of measurement provides information regarding transport of materials from the blood to brain, i.e. the luminal side of the brain capillary. In the present study, the rate constant of washout of valproate has been determined from measurements of brain valproate content at various times after a single intracarotid injection. The slope of this line (K_{test}) = 0.74 min^{-1}, is the rate constant of efflux of the test compound (i.e. valproate); and K_{ref} = 0.55 min^{-1}, the rate constant of efflux of the reference compound (tritiated water). The ratio of the K_{test}/K_{ref} is an indicator of the relative permeability of the antiluminal surface of the brain capillary; i.e., brain-to-blood permeability. Since this ratio 0.74/0.55 = 134%, it is apparent that brain-to-blood permeability of valproate is greater than that of blood-to-brain, which is suggestive of an active efflux mechanism for valproate. The asymmetric BBB transport of valproate may possibly be attributed to plasma protein binding effects, since no CNS binding in brain has been observed, but valproate is known to bind to serum protein. If valproate is bound to albumin as avidly as are long chain fatty acids, then partial mixing of the saline-borne drug with circulating rat plasma might render valproate partially unavailable for blood-to-brain transport. However, the binding of valproate by serum of rat or eight other species is relatively weak (e.g. freely dialyzable fraction = 5-88%, depending on the species) compared to the long chain fatty acid binding. Moreover, we find that the albumin-bound valproate is available for transport through the BBB. Therefore we conclude that the asymmetric BBB transport of valproate is not due to plasma protein binding effects. Polarity of the blood-brain barrier to many compounds has been described on the basis of in vitro studies of isolated brain capillaries, but to our knowledge this represents the first in vivo demonstration of blood brain barrier polarity.

234.2 ELECTRICAL IMPEDANCE CHANGES IN CEREBRAL ISCHEMIA RELATED TO INCREASES IN INTRACRANIAL PRESSURE. P. Ting*, H. Wagner, K. Kito*, T. Yamaguchi* and I. Klatzo. National Institutes of Health, Bethesda, MD 20205.

The purpose of this study was to observe the effects of focal ischemic brain edema upon cerebral extracellular space determined by impedance measurements. Cats were subjected to left middle cerebral artery(MCA) occlusion for 1 hr. Immediately after recirculation, 2% Evans Blue(EB) tracer was given IV for blood-brain barrier(BBB) evaluation. The cats were sacrificed between 6 and 42 hrs. following release of MCA occlusion. Cerebral electrical impedance(CEI) and regional cerebral blood flow(rCBF) were measured using a 5 platinum microelectrode array inserted into the ipsilateral caudate. During ischemia(rCBF=11±ml/100g/min), impedance rose to $\bar{x}$=211%. Immediately after release, CEI decreased but it was followed by a second rise to $\bar{x}$=176% within 15 hrs. of recirculation and this late rise was not accompanied by ischemia (rCBF=53±5ml/100g/min). A secondary rise was also observed in cats in which the MCA was permanently occluded with ischemia rCBF=±1ml/100g/min. All cats revealed extravasation of EB in ischemia areas. The secondary rise in CEI appeared to be related to increased intracranial pressure(ICP) induced by ischemic brain edema. To test this latter hypothesis, brain compression was produced by epidural balloon inflation. When the epidural pressure rose from a baseline value of 5± 3mmHg, to 26± 6mmHg, the CEI increased to 216% and rCBF dropped from a baseline value of 48ml to 25ml/100g/min. This study suggests that an increase in ICP itself can produce a reduction in extracellular space without lowering rCBF to critical ischemic values and that secondary rise of CEI in cerebral ischemia might be therefore related to compression of extracellular space due to increased tissue pressure induced by the development of edema.

234.3 ANTIBODIES TO BOVINE BLOOD-BRAIN BARRIER PLASMA MEMBRANES BIND TO ENDOTHELIAL CELL JUNCTIONS AND TO 46K AND 68K PROTEINS. W.M. Pardridge, J. Yang* and J. Eisenberg*. Dept. of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

Similar to epithelial barriers, brain capillary endothelial cells, which form the blood-brain barrier (BBB), have tight junctions and exhibit polarization of the luminal and anti-luminal membranes. In an attempt to begin elucidating the biochemical mechanism of brain endothelial membrane specializations, we initiated an immunochemical characterization of bovine brain capillary plasma membranes. Microvessels were obtained by a mechanical homogenization technique from either rat or bovine brain. The brain capillary plasma membrane fraction was prepared according to the method of Lidinsky and Drewes (J. Neurochem. 41, 1241, 1983). The plasma membranes were analyzed by SDS polyacrylamide gel electrophoresis (PAGE) and both the rat and bovine BBB plasma membranes had major bands of 68K and 46K molecular weight. The plasma membrane fraction was enriched in alkaline phosphatase, gamma glutamyl transpeptidase and insulin receptor binding activity. These plasma membranes from either rat or bovine brain microvessels were injected into rabbits and polyclonal antibodies were prepared. The rabbit antiserum against bovine BBB plasma membranes immunoprecipitated the 68K and 46K bands, whereas the rabbit antiserum prepared against the rat BBB plasma membranes immunoprecipitated the 68K protein in the bovine BBB plasma membrane fraction. Both the antirat BBB antiserum and the antibovine BBB antiserum diffusely stained bovine microvessels with an avidin/biotin/peroxidase histochemical technique. Since these microvessels have an intact basement membrane and diffuse binding of antibodies to the basement membrane may obscure antibody binding to the underlying plasma membranes, we prepared bovine endothelial cells using the method of Bowman et al (Ann. Neurol. 13, 396, 1983). These capillary endothelial cells were trypan blue negative and were positive for gamma glutamyl transpeptidase. The antirat BBB antiserum gave a faint speckled pattern in the avidin/biotin/peroxidase histochemistry, but the antibovine antiserum intensely stained the lateral margins of the bovine endothelial cells and the cell-to-cell junctions. The correlation of the SDS-PAGE immunoprecipitation studies and the immunoperoxidase studies suggests that a major bovine BBB plasma membrane antigen is the 46K protein. This protein appears to be asymmetrically distributed on bovine brain capillary endothelial cell membrane and possibly to endothelial tight junctions.

234.4 INHIBITION OF CHOROID PLEXUS Na^+-K^+ ATPase AND CEREBROSPINAL FLUID FORMATION. M.Pollay,E.Reynolds*,P.Tompkins*,A.Roberts* and A.Stevens*. Neurosurgery Laboratory, Univ. of Okla. Health Sciences Center, Oklahoma City, OK 73126.

The relationship between transport ATPase and volume flow into the cerebral ventricles was studied in rabbit and dog using a ventriculo-aqueductal and ventriculo-cisternal perfusion system respectively. The effect of various concentrations of ouabain (10^{-8} to 10^{-3}M) on cerebrospinal fluid (csf) formation was computed from the dilution of a non-diffusible marker in transit through the ventricular system. The degree of inhibition of ATPase in plexus was measured in vitro using a radiolabeled enzymatic method. The total amount of ATPase measured in rabbit choroid plexus was 14.7 moles/hr/kg dry weight with 26.3% (3.87) activated by Na^+-K^+ and the remainder by Ca^{++}/Mg^{++}. In the dog plexus the total, Na^+/K^+ activated, and the Ca^{++}/Mg^{++} activated enzyme activity was 16.9, 3.4 and 13.5 moles/hr/kg dog weight respectively. In the presence of 10^{-3}M ouabain, both rabbit and dog plexus transport ATPase (Na/K) was completely inhibited. When the cerebral ventricles were perfused with an artifical csf containing ouabain in a concentration of 10^{-4} to 10^{-3}M, the rate of intraventricular fluid formation fell 68% in the dog and 90% in the rabbit. These results indicate that a portion of intraventricular csf formation is cardiac glycoside insensitive and suggests that more than one mechanism is involved in the elaboration of this fluid. This view is compatible with the notion that there is a partial extrachoroidal source for the formation of intraventricular fluid utilizing a mechanism unrelated to transport ATPase. It is also possible, in part, that volume flow across the choroidal ependyma may not be secondary to local osmotic gradients produced by the active transport of sodium.

234.5 PROPERTIES OF GLUCOSE 6-PHOSPHATASE IN CEREBROVASCULAR ENDOTHELIUM. B.M. Djuricic* and M. Spatz. Laboratory of Neuropathology and Neuroanatomical Sciences, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205 USA.

Glucose 6-phasphatase (G 6-Pase; E.C.3.1.3.2) is detectable in the brain tissue but its role is debatable since the brain is neither a glyconeogenetic tissue or contains high glycogen reserves. The consideration of G 6-Pase involvement in the transfer of glucose across the blood brain barrier (BBB) represent the must attractive implicated function for this enzyme which activity is about 20 times higher in the isolated cerebral microvessels that in the brain parenchyma (538.0±63 and 28.0±5 mU mg^{-1} protein, respectively). Therefore, we investigated the biochemical characteristics of G 6-Pase in cerebrovascular endothelium using two models: isolated microvessels and pure cultures of cerebrovascular endothelial cells (4th - 6th generation) derived from the microvascular fraction of rat brain.

Glucose 6-P (G 6-P) was the best substrate among the tested sugar phosphates (glucose 1-P, erythrose 4-P and 4-P glycerate) in both cell types. Ribose 5-P gave the same response as glucose 6-P while fructose 6-P was a good substrate for the cultured cerebrovascular endothelium but not for the isolated microvessels.

The cerebrovascular endothelial G 6-Pase in contrast to that of the liver failed to phosphoryte glucose using carbamyl phasphate as donor. Kinetically a marked activation of G 6-Pase occured at high concentration of G 6-P (over 2 mmoles/l up to 25 mmoles/l). A biphasic response curve of the G 6-Pase activity was seen in the presence of either increased relative concentration of substrate or the amount of tissue enzyme. ATP as well as the non-hydrolyzable analogue adenyl (β,γ-methylene) diphosphate stimulated also the activity of endothelial G 6-Pase. The gel electrophoresis showed a single site of enzymatic activity corresponding to a single protein band irrespective of the tissue source.

The high concentration of G 6-Pase in the cerebrovascular endothelium, its kinetic activation pattern [(allo-) steric] distinctly different from other tissues are indicative of a specific role of this enzyme in the cerebral microvasculature compatible with the proposed participation of G 6-Pase in the glucose transpor across the BBB.

234.6 CEREBROVASCULAR TRANSPORT OF LARGE NEUTRAL AMINO ACIDS IN MATURE AND AGING RATS. Y. Takasato*, S. Momma* and Q. R. Smith. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20205.

Large neutral amino acids (LNAA) are transported across the blood-brain barrier by a facilitated mechanism which is stereospecific and saturable. Facilitated transport increases unidirectional influx of LNAAs by ∿20 fold, which may be necessary to sustain cerebral synthesis of proteins, peptides and some neurotransmitters. During the first 2 months of postnatal life in the rat, influx of LNAAs into the brain decreases ∿10 fold and there is a corresponding decline in the rate of cerebral protein synthesis (Banos et al., 1978). To determine whether significant changes in LNAA influx occur with senescence, we measured the concentration dependent uptake of cycloleucine, a nonmetabolized model LNAA, in young (3 mo) and aged (24 mo) rats.

In pentobarbital-anesthetized Fischer-344 rats, the right cerebral hemisphere was perfused with physiological saline or plasma, containing ^{14}C-cycloleucine, ^{3}H-inulin and 0-20 μmol/ml of unlabeled cycloleucine. After 60 s of perfusion, the rat was decapitated and samples from 6 brain regions and infusion fluid were analyzed for radiotracer content. The mean cerebrovascular permeability-area product (PA) was calculated for each cycloleucine concentration, and the Michaelis-Menten parameters, Vmax and Km, were determined from nonlinear regression analysis of the PA data. The apparent Km for cycloleucine influx from plasma was calculated from the PA for cycloleucine during plasma perfusion.

Cerebrovascular PA to cycloleucine decreased ∿30 fold in both young and aged rats as perfusate cycloleucine concentration increased, which is consistent with saturation of a transfer site. However, Vmax and Km did not vary significantly with age and equaled 9.5 ± 1.4 x 10^{-4} μmol/s/g and 0.28 ± 0.4 μmol/ml, respectively. Similarly, the apparent Km for cycloleucine transport from plasma, 4.5 ± 0.6 μmol/ml, did not change significantly between 3 and 24 months of age. Lastly, the plasma concentration of each of 9 LNAAs did not vary with age, except for threonine which increased by 53% at 24 months ($p<0.05$).

These data show that, contrary to some previous reports, cerebrovascular transport of LNAAs is maintained with age. If LNAA transport is coupled to brain protein synthesis, these findings suggest that the rate of cerebral protein synthesis does not decline between 3 and 24 months of age in the F-344 rat.

234.7 IONIC PERMEABILITIES OF THE ENDONEURIAL CAPILLARIES IN THE FROG SCIATIC NERVE. A. Weerasuriya* and S.I. Rapoport. (Spon. R.E. Taylor) Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, Maryland 20205.

Peripheral axons function within a specialised micro-environment, the endoneurial space, which is separated from other extracellular spaces by endoneurial capillaries and perineurium. The regulation of ionic concentrations within the endoneurium, and especially in the immediate periaxonal space, is essential for normal axonal functions. The absence of evidence for active transperineurial transport of Na and K and the low permeability of the perineurium to ions (Na permeability of 1.7×10^{-6} cm/sec) indicate the relatively passive role of the perineurium in defining and tightly limiting the endoneurial space. Using an in situ perfusion technique (Rapoport & Weerasuriya, Soc. Neurosci. Abstr., Vol 9, 162, 1983) we investigated the permeability-surface area product (PA) of endoneurial capillaries in sciatic nerves of Rana pipiens to ^{42}K, ^{22}Na and ^{36}Cl. The PAs of [14C] sucrose and ^{42}K and the ratios of these PAs were measured simultaneously and found to be independent of perfusate K concentration (0.1 mM to 10.0 mM) (see table below). The PAs of ^{42}K and ^{36}Cl when measured simultaneously in normal (2.5 mM K) Ringer were 39.1 ± 5.8 (S.E.M.) and $32.8 \pm 4.5 \times 10^{-5}$ sec^{-1} (n=12) respectively. The Cl/K permeability ratio (0.86 ± 0.02) was significantly different ($p < 0.02$) from the limiting conductance ratio (1.04) of these ions in free solution. The PAs of ^{22}Na and ^{42}K when measured simultaneously in normal Ringer were 21.1 + 4.6 and 30.1 ± 6.9×10^{-5} sec^{-1} (n=8) respectively. The K/Na permeability ratio (1.38 + 0.06) was not significantly different ($p > 0.05$) from the respective limiting conductance ratio (1.47). Thus, within the investigated range of perfusate K concentrations there is no evidence for facilitated transport of K by the endoneurial capillaries. Furthermore, the results suggest that K, Na and Cl traverse these capillary walls predominantly along a paracellular route which may be somewhat selective for cations.

[K]	n	[14C]sucrose	^{42}K	K/sucrose ratio
mM		PA (sec^{-1}) x 10^5		
0.1	16	7.2 ± 0.7	32.5 ± 3.9	4.43 ± 0.28
2.5	14	9.8 ± 0.8	42.9 ± 4.2	4.35 ± 0.23
4.7	9	9.9 ± 1.3	41.3 ± 6.7	4.02 ± 0.22
10.0	16	9.6 ± 0.8	39.7 ± 4.5	4.02 ± 0.28

234.8 POSITRON EMISSION TOMOGRAPHIC MEASUREMENT OF CEREBRAL BLOOD FLOW AND WATER PERMEABILITY WITH O-15 WATER AND C-11 BUTANOL. P. Herscovitch*, M.E. Raichle, M.R. Kilbourn* and M.J. Welch*. Washington University School of Medicine, St. Louis, MO 63110.

Tracers for the measurement of regional cerebral blood flow (CBF) using the Kety autoradiographic method in laboratory animals (Kety, Methods Med Res 8:228, 1960) or its adaptation to positron emission tomography (PET) in humans (Herscovitch, J Nucl Med 24:782, 1983) ideally should freely cross the blood-brain barrier. Tracers that are less than freely permeable, e.g. O-15 water, may underestimate CBF, especially at higher flows. Determination of this underestimation, in relation to flow measured with a freely diffusible tracer, permits the calculation of the extraction of the less diffusible tracer and, by application of the Crone-Renkin model, its permeability (Raichle, Adv Neurol 28:423, 1980). We have validated C-11 butanol as a freely diffusible, reference CBF tracer for PET, and used it in humans to determine the underestimation of CBF with O-15 water and the permeability of the brain for water. Our measurements of the brain permeability of C-11 butanol in baboons, using an intracarotid injection, external detection method, demonstrate that this tracer is freely permeable up to a CBF of at least 170 ml/(min•100g), and that it should accurately measure CBF when used with PET. To establish the validity of this assumption, we measured CBF with PET in baboons and compared it to CBF measured with a standard, intracarotid injection, residue detection method. The correlation was excellent [CBF(PET+butanol) =0.91 CBF(true) + 0.83, r=.97, n=9]. Finally, we measured CBF in humans using PET and both C-11 butanol and O-15 water. Our data indicate a modest 8% underestimation of average whole brain CBF with O-15 water. Using CBF data from cortical regions (which, because of the limited spatial resolution of PET, contain a mixture of gray and white matter) we obtained regional extraction values for water of 0.85 ± 0.04 (SD) and permeablity-surface area product measurements of 155 ± 12 ml/(min•100g). These results are similar to data obtained in primates using more invasive techniques and demonstrate for the first time the potential of PET to measure brain water permeability regionally, in vivo, in humans. The ability to make this measurement is of special importance in testing the hypothesis that regulation of brain water permeablity is a function of a central neuroendocrine system (Raichle, Acta Neuropath, Suppl VII:75, 1983).

234.9 DIFFERENTIAL EFFECT OF INTRACAROTID MANNITOL ON THE ENDOTHELIUM OF LARGE INTRACRANIAL ARTERIES AND CAPILLARIES IN DOGS. J. Godersky, T. Sasaki*, N. Kassell*. Div. of Neurosurgery, Univ. of Iowa, Iowa City, IA 52242

This study was designed to investigate if there was a difference in the response of the endothelium of large intracranial vessels compared to the brain capillaries, following internal carotid (IC) artery mannitol injection in dogs. The study consisted of 2 parts. Part one, 6 animals had 45 cc of 25% mannitol injected into the left IC over 30 seconds. Evans blue and I^{125} labeled albumin were injected prior to the mannitol. The dogs were sacrificed 30 minutes later. Blood brain barrier (BBB) breakdown to Evans blue occurred ipsilateral to the mannitol injection and a significant difference between right and left hemisphere I^{125} counts was noted. No difference was noted in I^{125} activity of the cavernous IC, intracranial IC, middle cerebral arteries on the 2 sides. No blue staining of the intracranial vessels was seen. Four additional dogs had mannitol injection into the common carotid artery. BBB breakdown was not seen and no difference in right and left I^{125} brain or vessel activity was noted.

Part two (5 dogs), morphology of the cerebral vessels was studied by transmission electron microscopy (TEM). The same procedure was used except horseradish peroxidase (HRP) was substituted for I^{125} labeled albumin and time to sacrifice was shortened to 10-20 minutes. Microscopic observation of the cerebral vessels demonstrated variable staining with HRP reaction products along the course of the vessel. When the staining was light, it was most pronounced at branch points, when it was dark the entire vessel was involved. The brain staining with reaction product occurred in the same distributions as that of the Evans blue. By TEM, HRP was seen in the intercellular and subendothelial spaces of the capillaries. Penetration of the HRP through the endothelium of the major vessels was less pronounced than in the capillaries and variable in extent. Various size vesicles and channels containing HRP were evident in the endothelial cells of the large vessels and occasionally HRP was present in the subendothelial space of these vessels. Endothelial disruption was seen inconsistently. Conclusion: Large vessel and capillary endothelium respond differently to hyperosmolar stress.

235.1 CEREBELLAR PATHWAYS IN THE CONDITIONED NICTITATING MEMBRANE RESPONSE. C.H.Yeo*, M.J.Hardiman* and M.Glickstein. MRC Unit on Neural Mechanisms of Behaviour, University College London, London WC1E 7JG, U.K.

Large cerebellar lesions abolish the conditioned nictitating membrane response (NMR) in rabbits but leave the unconditioned response to airpuff or shock intact. Lesions restricted to anterior parts of the interpositus nucleus are as effective in abolishing the conditioned NMR as complete cerebellectomy. Recently we found that small lesions restricted to the hemispheral portion of lobule VI (HVI) and sparing the underlying nuclei also abolish the conditioned NMR. In order to study the efferent and afferent connections of this small cortical area we injected wheatgerm agglutinated horseradish peroxidase (HRP) into HVI of four rabbits. After two days they were deeply anaesthetised and perfused. The brains were sectioned and reacted with tetramethyl benzidene to reveal orthograde and retrograde transport of HRP.

We found a dense projection to the anterior interpositus nucleus. The preterminal orthograde label was densest in that region of the nucleus which we have found to be necessary for NMR conditioning. There were retrogradely labelled cells in the lateral reticular, spinal trigeminal and pontine nuclei. One of the pontine cell groups was in the dorsolateral nucleus - an area known to receive auditory and visual input. We also found that a small group of retrogradely labelled cells in the most medial portion of the dorsal leaf of the principal division of the inferior olive and in the adjoining medial part of the dorsal accessory division. In cats, this area receives input from the spinal trigeminal nucleus and its cells respond to somatosensory stimulation of the face.

The anatomical results are consistent with the suggestion that pontine cells relay auditory and visual information related to the conditional stimulus (CS), via mossy fibres, to HVI. Olivary cells relay information about stimulation of the eye or paraorbital region related to the unconditional stimulus (US), via climbing fibres, to HVI. The association between CS and US might then be made at the level of the Purkinje cell in the cerebellar cortex.

235.2 BRACHIUM CONJUNCTIVUM AND RUBROBULBAR TRACT: BRAIN STEM PROJECTIONS OF RED NUCLEUS ESSENTIAL FOR THE CONDITIONED NICTITATING MEMBRANE RESPONSE J.W. Moore, M.E. Rosenfield* and A. Dovydaitis* Department of Psychology, University of Massachusetts, Amherst, MA 01003

The rubroblbar tract (RT) in rabbit to the level of the accessory abducens nucleus is described: Orthograde labeling of fibers of RT following horseradish peroxidase implants into red nucleus (RN) of 8 animals permitted ad hoc analysis of the effects of brain stem lesions of the rabbit's conditioned nictitating membrane (NM) response; 24 rabbits, trained to give NM CRs from both eyes, received unilateral lesions of the right pontine brain stem. Six of the 7 cases of post-lesion disruption of ipsilateral CRs involved either ipsilateral brachium conjunctivum or RT. URs were not affected. These findings, together with a reexamination of data from 2 related studies from this laboratory (Desmond, J.E. and J.W. Moore, Physiol. Behav. 28:1029-1033, 1982; Rosenfield, M.E. and J.W. Moore, Behav. Brain Res. 10: 393-398, 1983), strongly support the conclusion that an essential premotor component of the conditioned NM response is a doubly decussating circuit from the interpositus nucleus of the cerebellum (see Glickstein, M, M.J. Hardiman and C.H. Yeo, J. Physiol. (Lond.) 341: 30-31P, 1983; McCormick, D.A. and R.F. Thompson, Science 223: 296-300, 1984) to magnocellular RN via brachium conjunctivum and from RN caudally to the accessory abducens nucleus where motoneurons involved in the NM resonse are located. Desmond and Moore found 10 cases of CR disruption out of 30; Rosenfield found 11 disrupted cases out of 16: all involved some link in this circuit. Equally relevant to the circuit hypothesis are the 42 nondisrupted cases from the 3 studies. The lesion of 1 nondisrupted animal from the Desmond and Moore study included rostral portions of RT. None of the 5 nondisrupted cases from the Rosenfield and Moore study involved the circuit, and none of the nondisrupted cases of the present study involved the circuit. With only 2 clear counter instances out of 70 cases from the 3 studies, the phi correlation coefficient relating disruption of conditioned responding to lesions of the circuit is .94 ($p < .001$). The possible role of the supratrigeminal reticular formation (Desmond, J.E. and J.W. Moore, Brain Res. Bull. 10:765-773, 1983) in this learned behavior is also discussed.

235.3 WITHDRAWN

235.4 N^6-(L-PHENYLISOPROPYL)ADENOSINE RETARDS ACQUISITION OF THE CLASSICALLY CONDITIONED RABBIT'S NICTITATING MEMBRANE RESPONSE. L. Winsky, I. Gormezano, and J. A. Harvey. Depts. of Psychology and Pharmacology, The University of Iowa, Iowa City, Iowa 52242

The adenosine receptor agonist N^6-(L-phenylisopropyl) adenosine (L-PIA) has been identified as a potent psychoactive compound. In this study, the effects of L-PIA were examined in relation to acquisition of the conditioned nictitating membrane response. New Zealand albino rabbits were given subcutaneous injections of 0, 0.01, or 5.0 umol/kg of L-PIA prior to each of 10 daily conditioning sessions. Each session consisted of 60 trials composed of 30 tone and 30 light conditioned stimuli (CSs) paired with a 100 msec electric shock (3mA) unconditioned stimulus. Effects of L-PIA on acquisition were determined by the frequency of conditioned responses (CRs) and response latencies over the 10 daily sessions and by the number of trials required to reach the criteria of 1, 5, and 10 consecutive CRs. It was found that L-PIA significantly retarded CR acquisition to both the tone and light CSs at both doses examined with the greatest retardation at the 5.0 umol/kg dose. While all saline control rabbits (N=11) attained the criterion of 10 consecutive CRs (irrespective of CS modality), only 58% of those at the 0.01 umol/kg dose (7/12) and 27% of rabbits receiving 5.0 umol/kg (3/11) reached this criterion. In a separate experiment, the effects of caffeine base on CR acquisition were also examined. Overall, doses of 0.003, 0.01, 0.03, 0.10, and 0.30 mmol/kg of caffeine administered via subcutaneous injection did not affect CR acquisition.

The agonist activity of L-PIA has been associated with activation of A_1 adenosine receptors that produce decreases in brain levels of cAMP. Thus, the effects of L-PIA on CR acquisition may have been due to an effect on cAMP levels. The adenosine receptor antagonist activity of caffeine is less specific since caffeine appears to have similar affinities for both the A_1 and A_2 receptor subtypes. In contrast to A_1, activation of the A_2 receptors has been associated with stimulation of cAMP production. The absence of any effect of caffeine on CR acquisition may somehow be related to its less specific effects on the cAMP system.

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235.5 STRYCHNINE INCREASES ACOUSTIC STARTLE AMPLITUDE BUT DOES NOT ALTER SHORT-TERM OR LONG-TERM HABITUATION. J.H. Kehne* and M. Davis. (SPON: C.A. Sorenson). Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508.

Theories of habituation have included an accruement of postsynaptic inhibition as one possible mechanism underlying response decrement following repetitive stimulus presentation. The present study used the glycine antagonist strychnine (1.0 mg/kg, ip, 10 min pretreatment) to investigate the involvement of glycinergic neurons in the development and/or expression of short-term (within-session) habituation (Experiment 1) and long-term (between-session) habituation (Experiments 2 and 3) of the acoustic startle response in rats. Over a range of eliciting stimulus-intensities (95, 105, and 115 dB) and inter-stimulus intervals (3, 7, 13, and 27 sec), strychnine markedly increased startle amplitude relative to water injection whereas it failed to attenuate the rate of within-session habituation. Experiment 2 assessed the effect of strychnine on the expression of between-session habituation of startle. Rats that were exposed to daily sessions of startle-eliciting stimuli for four days and then tested on the fifth day showed lower overall levels of startle amplitude relative to rats that had not received prior habituation training. Strychnine injected prior to the test session again increased startle amplitude, but did not block the expression of between-session habituation. Experiment 3 tested the possibility that glycinergic neurons might be involved in the development (rather than the expression) of between-session habituation. Rats injected with either strychnine or water prior to each of three daily habituation training sessions and subsequently tested on Day 4 showed similar between-session habituation relative to untrained rats that had received daily injections in the animal room. In summary, strychnine increased startle amplitude without affecting either within-session or between-session habituation of acoustic startle. These results emphasize the need to discern between drug effects on response amplitude per se and effects on response habituation. Furthermore, the data indicate that an accruement of inhibition in glycinergic neurons does not explain either within-session or between-session habituation of acoustic startle in rats. These results are discussed in light of current theories of habituation.

235.6 REGIONAL METABOLIC ACTIVATION IN CORTEX, THALAMUS, AND HIPPOCAMPAL FORMATION DURING CLASSICAL CONDITIONING: A 2-DEOXYGLUCOSE STUDY. F. González-Lima and H. Scheich. Dept. of Anat., Ponce Sch. of Med., Ponce, PR 00732 and Zool. Inst., Tech. Univ., 6100 Darmstadt, West Germany.

The (^{14}C) 2-deoxyglucose (2DG) autoradiographic method was used to examine the metabolic activity in all telencephalic and diencephalic structures of the rat brain during and after conditioning. A trial was made of a 4-5 KHz frequency modulated tone (CS, 2s, 60dB) paired with midbrain reticular stimulation (US, 0.5s, 40Hz, 300-600 μA) with the US shock overlapping the last 0.5s of the tone CS. The conditioned response was a rapid bradycardia elicited by the US. Alert rats were injected with 2DG (i.p. 18 μCi/100g) and placed in a sound-proof chamber. Six groups of rats were subjected during 90 min. to a given stimulus treatment: 1) the tone CS before conditioning, 2) the US alone, 3) the paired CS-US, 4) the tone CS after conditioning, 5) the unpaired US and CS, and 6) no stimulation. The major findings consisted of specific patterns of metabolic activation evoked during conditioning (group 3) in the auditory cortex, prefrontal cortex, posterior parietal cortex, medial thalamus, lateral habenula, and hippocampal formation. Of these only the hippocampal formation showed a significant increase of 2DG uptake to the tone CS after conditioning (group 4). 2DG was concentrated in a central band along the sides of the hippocampal fissure which corresponded to the molecular layers. Only this neuropil band of greater metabolic activity showed the learning-related change. A columnar organization was well-defined in the posterior parietal cortex of rats subjected to CS-US pairing. Significant 2DG changes in the parietal cortex and lateral habenula were found only in the right hemisphere. While the learned value of the CS seemed to be represented mainly in the hippocampal formation, the other structures activated during conditioning may be associated with functions involved in the formation of a memory trace (i.e. directed attention, motivation, temporal integration). The changes revealed by 2DG may provide a useful approach to the problem of localization in two ways. First, by identification of structures (or subsystems) that participate in a learned response; and second, by identification of cellular substrates (i.e. synaptic elements) underlaying plasticity in suitable structures with clearly defined cytoarchitecture and conections (eg. hippocampus). (Supported by NIH-MBRS grant RR08067; by DFG-SFB45, and by the Humboldt-Stiftung).

235.7 INTRACELLULAR CONSEQUENCES OF US PRESENTATIONS IN CELLS OF THE MOTOR CORTEX OF CATS. D. Birt and C.D. Woody. Depts. of Anatomy and Psychiatry, UCLA Med. Center, Los Angeles, CA 90024

Presentation of unconditioned stimuli such as glabella tap produces an initial increase in unit activity in cells of the motor cortex, followed (20-40 ms) by a pronounced decrease in activity and then a second period of increased activity (Birt and Woody, Soc. Neurosci. Abstr., 1983). The decrease in activity is characteristic of stimuli which serve as effective USs for classical conditioning but not stimuli used as CSs (e.g., 65-70 db clicks).

We have now studied intracellular consequences of glabella tap-US presentation in 81 cells of the motor cortex. Most cells showed short latency (<20 ms) depolarizing potentials (EPSPs) followed by hyperpolarizing potentials (IPSPs) occurring at latencies of 20-40 ms. The latter were typically of small magnitude and were often obscured by superimposed continuing EPSPs. In 35 of these cells hyperpolarizing currents were injected through the recording electrode. Typically, this increased the size of the short latency EPSP. Some IPSPs decreased in magnitude and then reversed with increasing levels of hyperpolarization in a manner similar to IPSPs recently demonstrated in hippocampal pyramidal cells (Nicoll and Alger, Science, 1981). However, in nine cells, IPSPs which increased in size with increasing hyperpolarization were observed. These IPSPs resembled IPSPs recorded from cells of the motor cortex following pyramidal tract (PT) stimulation (Bindman et al., Soc. Neurosci. Abstr., 1982). The latter IPSPs are associated with a decrease in membrane conductance.

Both glabella tap and PT stimulation result in long-term increases in the excitability of neurons of the motor cortex (Brons and Woody, J. Neurophysiol., 1980). PT stimulation has also been used successfully as a US for conditioning (O'Brien et al., J. Comp. Physiol. Psychol., 1977). The finding that two different stimuli which can serve as USs for conditioning both produce long-term excitability increases in neurons of the motor cortex and produce similar EPSP-IPSP sequences raises the possibility that sequences of the type observed could be importantly involved in producing the long-term excitability increases. (Supported by NIH and AFOSR)

235.8 POSITIVE REINFORCEMENT DURING INSTRUMENTALLY CONDITIONED BEHAVIOR CAUSES PHASIC RANDOMIZATION OF NEURONAL FIRING PATTERNS IN THE FELINE CENTRUM MEDIANUM. T.J. Marczynski and L.L. Burns*, Department of Pharmacology, Univ. of Illinois College of Med., Chicago, Illinois 60612.

The mechanisms by which biological rewards influence the function of the thalamic association nuclei are poorly understood. Elimination of perseverative tendencies, learning and novel behavioral modes can only be achieved after the previously established and dominant functional connectivities between the association neuronal ensembles had been temporarily disrupted. The "behavior" of models of the central nervous systems support this notion (Nicolis et al. Biol. Cybernet., 17:183,1975). Hence, our working hypothesis was that positive reinforcement would decouple the dominant connectivities between the centrum medianum and other systems and that this process can be quantified by studying the statistical distribution of neuronal firing patterns. In 15 freely moving cats bearing microelectrodes in the centrum medianum (CM), temporal patterns in spike trains were analyzed in records that conincided with 5 sec postreinforcement periods and in records that coincided with attentive wakefulness (AW). The firing patterns were analyzed using a non-parametric method based on relative relations between sequential spike intervals and the distribution of patterns composed of 4,5, and 6 inequality signs. The deviations of pattern occurrences from the random model were quantified using chi square statistics (Marczynski et al. Brain Res. Bull.,8:565,1982; Int. J. Bio-Med. Comput.,14:463, 1983). A total of 44 neurons were analyzed, and the results showed that the distribution of patterns during AW strongly deviated from the random model (df=1364; χ^2=1581; P<.0001) while the distribution of patterns during the postreinforcement periods closely followed the random model (df=1364; χ^2=1247; P>.4). - Supported by PHS MH 8396.

235.9 PROJECTIONS FROM THE MEDIAL GENICULATE NUCLEUS TO AN ARCHI-NEOSTRIATAL FIELD MEDIATE AUDITORY FEAR CONDITIONING. J.E. LeDoux, A. Sakaguchi, J. Iwata, and D.J. Reis, Lab. Neurobiol. Cornell U. Med. Coll. NY, NY 10021

Auditory fear conditioning in rats is abolished by lesions of the inferior colliculus and medial geniculate (MG) but not of auditory cortex (LeDoux et al, J. Neurosci. 4: 683-696, 1984). We have therefore sought to identify subcortical targets of MG efferents and to evaluate their role in emotional conditioning.

Subcortical efferents of MG in rats were defined using anterograde and retrograde HRP tracing. Following microinjection of WGA-HRP (7-10 nl) in MG, anterograde transport was observed in the ventromedial hypothalamus (VMH), subparafascicular thalamus (SPF), and in a striatal field (STR), involving the portions of the caudal neostriatum (caudal third of caudate-putamen, CP) and and the underlying archistriatum (central and lateral amygdala), confirming our earlier observations (LeDoux et al, ibid.). Injection of VMH (n=2) retrogradely labeled cells in the peripeduncular region ventral to MG. Injection of SPF labeled the nucleus of the optic tract, dorsal to MG, and incosistently the medial MG. Injections into the archistriatal (n=2) or neostriatal (n=4) aspects of STR resulted in dense labeling of cells in the medial and dorsal MG.

Lesions were placed unilaterally in MG and in the contralateral VMH (n=6), SPF (n=6), STR (n=6), or anterior CP (n=4). Controls were unoperated (n=8) or received unilateral MG lesion alone (n=6). After 14 days the animals were classically conditioned (conditioned stimulus, CS: 800 Hz, 82 db, 10 sec pure tone; unconditioed stimulus (US): 2.0 mA, 0.5 sec, d.c.) and implanted with an arterial cannula for recording mean arterial pressure (MAP). Increases in MAP and the suppression of exploratory activity (as indicated by the duration of freezing) by the CS were measured.

The CS elicited elevations in MAP (16±3 mmHg) and induced freezing (90±15 sec) in unoperated controls. Unilateral lesion of MG alone or in combination with the contralateral VMH, SPF, or rostral CP did not affect responses. Lesions of MG and the contralateral STR reduced MAP by 67±1% ($p<.01$) and freezing by 68±1% ($p<.01$). Lesions involving the amygdala and caudal caudate had the same effects. Lesions of MG and the ipsilateral STR had no effect.

These findings demonstrate that the medial and dorsal regions of MG project to a striatal field involving the posterior neostriatum and dorsal amygdala and that interruption of these projections disrupts auditory fear conditioning. We conclude that auditory fear conditioning is mediated by projections terminating in or passing through STR. Supported by PHS grant HL 18974.

235.10 MULTIPLE RESPONSE DIMENSIONS IN PASSIVE AVOIDANCE BEHAVIOR: III. ASSESSMENT OF NEUROTOXICANT-INDUCED MEMORY DYSFUNCTION. C.F. Mactutus, Lab. Behav. Neurol. Toxicol., NIH-NIEHS, Research Triangle Park, NC 27709.

Behavior in a passive avoidance test is composed of inhibitory and vacillatory response components which are differentially affected by length of retention interval, prior training experience, and a high dose of d-amphetamine (Mactutus & Wise, 1984a). Administration of noncontingent footshock, a putative reactivation treatment, prior to a retention test also differentially affects inhibitory and vacillatory behavior (Mactutus & Wise, 1984b). We similarly examined retention of a passive avoidance response following administration of chlordecone, a neurotoxicant which alters membrane excitability and is suspected of impairing memory.

Fischer-344 rat pups were administered a single s.c. injection (20 µl) of either corn oil or of 1 mg/10 g chlordecone dissolved in corn oil on postnatal day 4. Body weights weights were slightly, but significantly, depressed on days 14 (10.5%) and 21 (9.5%) by the chlordecone exposure. On postnatal day 18, half of the pups were trained in a one-trial passive avoidance task, the other half received a comparable, but noncontingent, footshock in a neutral environment. One-half of each group of animals were tested after a 1-day retention interval, while the remaining animals were tested after a 7-day retention interval. Seven dependent measures were collected and subjected to principal components analysis to remove redundancy and identify the response dimensions underlying the behavior. Chlordecone had no effect on training response latencies. As previously observed, test behavior was composed of inhibitory and vacillatory response dimensions, and these dimensions were differentially affected by length of retention interval and prior training experience. Chlordecone did not affect inhibition or vacillation at the 1-day retention interval in either trained animals or noncontingent footshock controls. After the 7-day retention interval, however, chlordecone-exposed animals demonstrated significantly less inhibition than vehicle-injected controls and their inhibitory behavior was not distinguishable from that of noncontingent footshock controls. Vacillatory behavior was not affected by the neonatal chlordecone exposure after the 7-day retention interval. Collectively, these observations indicate that chlordecone may selectively impair the inhibitory component of passive avoidance behavior, and further support the utility of employing a multivariate approach for passive avoidance behavior when investigating potential memory dysfunction.

235.11 REVERSIBLE INACTIVATION OF THE HIPPOCAMPUS PREVENTS RETRIEVAL. Douglas C. Smith and James Talman* Developmental Biopsychology Program and Medical School, Southern Illinois University- Carbondale, IL. 62901.

Lidocaine is a local anesthetic which, when injected into the brain, completely and reversibly inactivates all neural activity in the area affected. Last year at this meeting, evidence was presented that post-trial bilateral injections of lidocaine into the hippocampus (HC) produced deficits in both short-term and long-term memory. We now report that lidocaine injections into the HC prior to a 24 hr retention test results in a complete retrieval failure.

In a one-trial inhibitory avoidance task, 30 Long Evans rats received a 1.5 ma, 1.0 sec shock immediately upon entering the dark compartment from the lighted side of a two compartment alley. Two experimental groups consisted of 10 animals each. For one group, 1 µl of lidocaine was injected into each HC within 5 sec after the learning trial and were tested 24 hr later (group PL). For the other group, bilateral HC injections were delayed until one minute before the 24 hr retention test (group PR). The 10 control rats had HC cannulae implanted and the same training and testing procedure but received no lidocaine (group SO). When tested 24 hrs after the training trial, group SO had a median latency to enter the dark of 300 sec (ceiling), demonstrating complete retention. For group PL, the median latency to enter the dark was 51 sec indicating that bilateral injections of lidocaine into the HC following the training trial interfere with consolidation of the learned event. This is true despite the fact that the lidocaine had worn off and the HC had been functioning normally for around 22 hrs prior to the retention test ($U=12.3$, $p<.05$). For group PR, the median latency to enter the dark was 8.3 sec. Compared to SO control rats, lidocaine administered to the PR rats prior to the retention test produces significant deficits in the ability to retrieve a previously learned inhibitory avoidance task ($U=0$, $p<.001$).

From these data it is concluded that the technique of reversible inactivation of neural tissue via lidocaine injections has allowed us to dissociate two processes which have confounded all memory research based on the lesion technique. Namely, consolidation vs retrieval. Further, such reversible inactivation of the HC produces deficits in consolidation when given shortly after the training trial and these deficits are apparent with the HC being completely functional at the time of testing. In addition, these data demonstrate that the HC must be operational in order for the rat to retrieve a learned inhibitory avoidance task.

235.12 COMPARISON OF THE TIME COURSE OF DEVELOPMENT OF AMNESIA IN MICE PRODUCED BY ANISOMYCIN AND OUABAIN. T.A. Patterson, S.J.Y. Mizumori, M.R. Rosenzweig and E.L. Bennett. Dept. of Psychology and Melvin Calvin Lab., University of California, Berkeley, CA 94720.

It has been suggested that different stages of memory formation have different cellular bases. Gibbs and Ng (1977) proposed that, in chicks, formation of short-term, intermediate-term and long-term memory are dependent, respectively, on K+ conductance, Na+/K+ ATPase activity and protein synthesis. The extent to which studies with the chick can be generalized to rodents was assessed in the following experiments.

Male CD-1 mice were trained in a one-trial, step-through passive avoidance task. Intrahippocampal injections of the Na+/K+ ATPase inhibitor ouabain (25 ng, 0.5 µl per side) were administered under light anesthesia. It was found that ouabain was effective in producing amnesia (tested at two days after training) when injected up to six hours before training and three minutes after training. Amnesia did not develop in animals injected with ouabain 24 hours before training or if injection was delayed for ten minutes or longer after training. When ouabain was administered three minutes after training, mice tested 15 minutes after training showed good retention. Ouabain-injected mice tested 30 minutes post-training or later were amnesic. Doses of ouabain less than 25 ng did not produce amnesia, while doses of 50 ng or greater resulted in adverse behavioral side-effects. Injection of 25 ng produced little or no side-effects. Biochemical experiments indicate that ^{3}H-ouabain concentration remains high in the mouse brain for at least six hours.

Experiments with intrahippocampally injected anisomycin, a protein synthesis inhibitor, indicate that anisomycin (80 µg, 0.5 µl per side) is effective in producing amnesia when injected 45 minutes before training, but not when injected after training. Pretraining injections of anisomycin produced amnesia which developed by 90 minutes post-training.

The different time courses of development of amnesia after ouabain and anisomycin treatment provide evidence for two of the three stages of memory formation proposed by Gibbs and Ng. The exact time courses differ between their studies and ours, but it is not yet known whether differences in species of subjects or of task is chiefly responsible.

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235.13 SUBICULAR LESIONS AND DISCRIMINATIVE NEURONAL ACTIVITY IN THE CINGULATE CORTEX AND ANTEROVENTRAL THALAMUS DURING LEARNING IN RABBITS. M. Gabriel, S. P. Sparenborg, N. Stolar*, D. Ragsdale* and S. Klein*. Dept. Psychol., Univ. Illinois, Champaign, IL 61820.

Greater neuronal discharges to a CS+ (a tone initiated 5 sec. in advance of a footshock US) than to a CS- (a tone predicting no US), develop during acquisition of discriminative (locomotory) avoidance behavior in rabbits. The activity develops sequentially, first in the anterior cingulate cortex [areas 24 and 32], next in the medial dorsal thalamic nucleus and the posterior cingulate cortex [area 29], and finally, as asymptotic discriminative performance is attained, in the anteroventral thalamic nucleus (AVN) [Gabriel et al, Science, 208: 1050, 1980; Orona & Gabriel, Brain Research, 263: 295, 1982]. Damage in area 29 or AVN impairs performance of the well-learned behavior, not its acquisition [Gabriel et al., Behavioral Neuroscience, 97: 675, 1983; Neurosci. Abst., 1984]. Acquisition is blocked by anterior cortical damage [Lambert & Gabriel, Neurosci. Abst. 86.19: 318, 1982]. Thus, the anterior and posterior cortices and their related thalamic nuclei contribute respectively to original acquisition and performance of the well-learned behavior. Recent studies of the effects of AVN damage have shown the thalamic afferent to be the principal source of CS-elicited excitation of area 29 neurons [Gabriel et al., op cit., 1983]. To examine the contribution of subicular afferents bilateral electrolytic lesions of the dorsal subicular complex, and implantation of microelectrodes in area 29 and AVN were performed in 10 rabbits. Controls (N=11) underwent surgery for implantation of recording electrodes, but sustained no lesions. Following recovery, discriminative training to criterion, overtraining, extinction, and reacquisition were given. As in past studies, discriminative CS-elicited discharges occurred in area 29 in the controls, during the initial and later training sessions. However, in subjects with lesions the discharges were attenuated and the early developing discriminative effect was absent. In contrast, CS-elicited firing was enhanced, and discriminative discharge acquisition accelerated in the AVN in subjects with lesions. These data suggest that subicular afferents support the early development and maintenance of discriminative activity in area 29, but they suppress neuronal firing and retard the development of discriminative activity in the AVN.

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235.14 DETOUR PROBLEM-SOLVING BEHAVIOR IN RATS WITH EARLY LESIONS TO THE "GENERAL LEARNING SYSTEM." R. Thompson, D. Harmon* and J. Yu. Dept. Physical Medicine and Rehabilitation, University of California Irvine Medical Ctr., Orange, CA 92668 and Fairview State Hosp., Costa Mesa, CA 92626.

The "general learning system" (GLS), an ensemble of neural structures conceived to play a significant role in the acquisition of a broad spectrum of laboratory tasks, of the rat brain has been proposed to comprise a number of brainstem regions, including those occupied by the globus pallidus (GP), substantia nigra (SN), median raphe (MR) and pontine reticular formation (PRF). This proposal is based on the findings that damage to any one of these regions in weanling (Thompson, R. and Yu, J., Physiol. Psychol., 11, 225, 1983) or adult (Thompson, R. Physiol. Psychol., 10, 293, 1982) rats leads to deficient learning of both visual and nonvisual discrimination habits. One of the questions arising from these data is whether the disruptive effects of lesions to the GLS are restricted to simple associative learning or whether these effects extend to more complex kinds of learning.

This study was done to assess the effects of selective lesions to the GP, SN, MR and PRF in weanling rats on subsequent acquisition of three separate "climbing" detour problems--mounting a raised platform, entering an elevated cylinder and climbing a ladder to gain access to the goal box containing food and water. Errors consisted of passing beyond the raised platform, opening of the cylinder and ladder. Earlier findings suggest that performance on Trial 1 is sensitive to a cognitive function that is different from that concerned with simple associative learning, as measured by performance on Trials 2-5.

Lesions to any one of these structures impaired performance (relative to that of sham-operated controls) on Trial 1 as well as on Trials 2-5 on all three problems. Nonspecific effects arising from brainstem damage cannot readily account for these results since rats with large lesions to the lateral portions of the brainstem at ponto-mesencephalic levels did not exhibit impairments on Trial 1 or Trials 2-5 on these problems. These findings provide further support for the notion that the GP, SN, MR and PRF are components of the rodent's GLS.

(Supported by a grant from the Rehabilitation Center for Brain Dysfunction, Inc.)

PAIN: CENTRAL PATHWAYS II

236.1 CAT NASAL RECEPTORS INVOLVED IN PROTECTIVE REFLEXES HAVE NOCICEPTIVE INPUT TO TRIGEMINAL NUCLEUS CAUDALIS. G.E. Lucier and R. Egizii*. Department of Medical Physiology, University of Calgary, Calgary, Alberta T2N 1N4, Canada.

The ethmoidal nerve innervates the anterior portion of the nasal mucosa and constitutes the afferent limb of several upper respiratory tract protective reflexes. Previous anatomical studies (Lucier and Egizii, Proc. of IUPS, 25:123.30, 1983) showed that ethmoidal afferents projected to all regions of the spinal trigeminal nucleus. The purpose of this study was to characterize the nature of the ethmoidal inputs onto cells in the subnucleus caudalis.

Adult cats were anaesthetized with alpha chloralose. Two tracheal cannulae were inserted, one to provide access to the lungs for ventilation and a second to allow air and test odorants to be pulled through the upper airway. Arterial blood pressure and end tidal CO2 were monitored. Bipolar stimulating electrodes were placed on the ethmoidal, vagus (X), hypoglossal (XII), glossopharyngeal (IX), and superior laryngeal (SLN) nerves as well as in the ipsilateral maxillary canine tooth pulp. During recording, all animals were paralyzed with gallamine triethiodide and artificially ventilated. The activity of functionally identified single neurons in nucleus caudalis was recorded using tungsten microelectrodes.

The inputs to over 200 caudalis neurons which had a discrete trigeminal receptive field were studied. Of these, those that received input from the ethmoidal nerve could be divided into two categories: neurons receiving short latency ethmoidal input (2-8 msec) and neurons receiving long latency ethmoidal input (10-30 msec). Neurons which received short latency input often received inputs from SLN and XII, but not from tooth pulp. These short latency units also received input from non-noxious stimuli (light touch, air puffs, etc.). One interesting distinguishing feature between the two groups was that the neurons having long-latency input also received input of a nociceptive nature from other trigeminal divisions (i.e. tooth pulp stimulation, noxious radiant heat applied to the face). These units occasionally had inputs from SLN and XII. They could also be stimulated by a non-electrical noxious ethmoidal input (i.e. ammonia vapour). Supported by the Canadian MRC.

236.2 THE DISTRIBUTION OF SPINAL AFFERENTS AND TRIGEMINOSPINAL EFFERENTS WITHIN TRIGEMINAL NUCLEUS INTERPOLARIS AND ORALIS. K. D. Phelan and W. M. Falls. Department of Anatomy, Michigan State University, East Lansing, MI 48824-1316.

The Spinal Trigeminal Nucleus (STN) gives rise to extensive projections to the spinal cord. Lesion studies indicate that STN also receives a spinal afferent input. However, the precise distribution of spinal afferents within STN and their relationship to locations of trigeminospinal projection neurons has not been described. In the present study, anterograde and retrograde transport of HRP and anterograde transport of tritiated amino acids were used to define and compare the location of spinal afferents to the location of trigeminospinal projection neurons within trigeminal nuclei interpolaris (Vi) and oralis (Vo) of the adult rat. Following unilateral injections into upper cervical segments, two main spinal afferent recipient zones are labeled within ipsilateral Vi and Vo. In transverse sections, one zone is a wedge-shaped region in the dorsolateral one-third of STN, located adjacent to the spinal trigeminal tract (SVT), which extends along the entire length of Vi and into caudal Vo. Spinal afferents to this area appear as a ventrolateral extension of spinal afferents to cuneate and external cuneate nuclei. Some spinal projecting neurons with 15-30μm somata occur in this area, although the majority of cells located here are trigeminothalamic projection neurons. The second zone is a narrow region (up to 120μm wide) lying adjacent to the ventrolateral two-thirds of the medial border of SVT along the entire length of Vi and Vo and extending into the trigeminal main sensory nucleus. Afferents within this zone are continuous with spinal afferents in the ventromedial reticular formation. In addition, spinal afferents in this ventrolateral zone extend into cerebellar projecting regions of the interstitial nucleus of the SVT. Many clusters of spinal projecting neurons with ∿15μm somata occur in this ventrolateral area as do numerous trigeminocerebellar neurons. In summary, this study demonstrates that spinal afferents project principally to two restricted zones bordering SVT in ipsilateral Vi and Vo. The smaller trigeminospinal neurons are primarily located within these zones and may receive a greater spinal influence than the larger trigeminospinal cells located in ventrolateral portions of Vi and Vo. In addition, spinal afferents in these zones may also influence known trigeminothalamic as well as trigeminocerebellar projection cells. Supported by N.I.H. Grant DE 06725.

236.3 RESPONSES OF HAMSTER SUPERIOR COLLICULUS NEURONS TO GRADED TACTILE, THERMAL, AND NOXIOUS STIMULI. M.A. Larson* and B.E. Stein (SPON: K. Corley), Dept. of Physiology & Biophysics, Medical College of Virginia, Richmond, VA 23298

There are many somatosensory cells in the hamster superior colliculus (SC): some respond to innocuous tactile stimuli and others either preferentially, or only, to noxious stimuli (Stein & Dixon, 1978). Yet, there is little quantitative data describing the responses of these cells. We sought to provide such data by relating stimulus intensity to impulse frequency using innocuous and noxious (mechanical and thermal) stimuli.

Fifty-nine somatosensory SC cells were studied in urethane anesthetized hamsters. Cells were characterized by responses to: a) controlled mechanical stimuli, and b) graded 5-s temperature shifts from a baseline of 35°C then stepped to 43°C and in 2° steps to 51°C.

Most (53%) were low threshold (LT), had force thresholds less than 1 g and adapted rapidly to maintained stimuli. Noxious pinch or heat (45-51°C) did not produce higher frequencies, or number of impulses, per response and these LT cells did not respond to thermal stimuli. A second and smaller population of cells (19%) responded to gentle mechanical stimulation with low impulse frequencies, to moderate-intensity stimulation with higher impulse frequencies, and to noxious pinch and heat (45-51°C) with the highest frequencies. The intensity-response frequency profiles of these wide dynamic range (WDR) neurons were linear, most WDR neurons adapted slower than did the LT Neurons and their receptive fields were larger. A third population (25%) was activated only by moderate or high intensity mechanical stimulation and responded maximally to pinch with toothed forceps. Receptive fields of these 'specific nociceptive' cells were smaller and their borders more distinct that WDR units, but of the seven cells that responded to thermal stimuli their responses were similar to WDR neurons by being linearly related to increases in skin temperatures from 43°-51°C. Two cells (3%) exhibited a complete suppression of spontaneous activity when either gentle mechanical stimuli and/or noxious pinch were presented.

These experiments demonstrated that somatosensory SC cells are not only capable of signalling the presence and location of a stimulus, but its intensity as well. Both WDR and specific nociceptive cells exhibit intensity coding capabilities though their functional ranges dffer significantly. Supported by a grant from the Jeffress Foundation.

236.4 NEURONAL RESPONSES IN ROSTRAL TRIGEMINAL BRAINSTEM NUCLEI OF MACAQUE FOLLOWING CHRONIC TRIGEMINAL TRACTOTOMY. R.F. Young and K.M. Perryman* UCLA School of Medicine, 10833 Le Conte Avenue, Los Angeles, California 90024

Unilateral trigeminal tractotomy was carried out in five young adult Macacca fascicularis monkeys. The animals had been trained previously to perform a behavioral shock avoidance task in response to presumably painful electrical stimulation of dental pulp and cutaneous electrical stimulation of facial skin. Tractotomy produced an elevation in the stimulus strength which elicited escape behavior when facial skin was stimulated, but not when the tooth pulp was stimulated.

Unit activity, evoked by electrical stimulation of the tooth pulp and facial skin, as well as innoccuous and noxious mechanical stimulation of orofacial regions, was recorded from neurons in the trigeminal nuclei principalis, oralis and interpolaris 8-12 weeks after tractotomy. Thirty-four units contralateral and fifty units ipsilateral to the tractotomy were studied. Thirty-six of the units were low threshold mechanoreceptors, forty-six were wide dynamic range units and six were nociceptors. No statistically significant differences between the populations of neurons ipsilateral and contralateral to the tractotomies were found for size or location of peripheral receptive fields, latencies, thresholds, mean firing densities or responsiveness to the various forms of stimulation. The behavioral results suggest that trigeminal relay neurons rostral to the obex are able to signal dental pain sensation and not surprisingly the physiological studies confirm that the firing patterns of such neurons is unaffected by tractotomy. The physiological studies demonstrate that the firing patterns of relay neurons in trigeminal brainstem nuclei rostral to the obex are also not affected by tractotomy. The cutaneous facial analgesia observed after tractotomy thus appears to be due to deafferentation of relay neurons in trigeminal nucleus caudalis rather than to interruption of intratrigeminal relays between the caudal and rostral nuclear groups.

Supported by Grant DE 05208 from the National Institute of Dental Research.

236.5 **PHRENIC NERVE STIMULATION: ITS EFFECT ON THE ACTIVITY OF T_2-T_5 SPINOTHALAMIC TRACT CELLS IN THE PRIMATE.** R.D. Foreman, W.S. Ammons, and N.-M. Girardot*. Univ. of Oklahoma HSC, Dept. Physiology & Biophysics. Oklahoma City, OK 73190.

The phrenic nerve is thought to contain primarily motor fibers that innervate the diaphram. Anatomical evidence in the rat indicates that 43% of the nerve is sensory (Langford and Schmidt, Anat. Rec. 205:207-213, 1983). An anecdotal statement in a review on hepatic nerves raises the possibility that the phrenic nerve contains hepatic nociceptor fibers. (Laut, Can. J. of Physiol. & Pharm. 58:105-123, 1980). These reports coupled with our interest in visceral pain and the close proximity of the nerve to the vagus nerve led us to examine the effects of phrenic nerve activity in spinothalamic tract (STT) cells of 5 monkeys (Macaca fascicularis) anesthetized with α-chloralose (60 mg/kg). STT cells were obtained in the left gray matter by antidromically activating their axons in the ventral posterior lateral nucleus of the thalamus and the medial thalamic nucleus in or near the centralis lateralis. After a STT cell was isolated, cardiopulmonary sympathetic afferent fibers were electrically stimulated and the somatic fields were determined. All cells received viscerosomatic convergence. Hook electrodes were placed around the phrenic nerve either near the heart or the diaphragm. The nerve usually was transected distal to the stimulating electrodes. Three STT cells increased their discharge rate from 6 spikes/s to a peak activity of 18 spikes/s during phrenic stimulation (20V, 1 ms, 20 Hz). Phrenic stimulation decreased activity in 3 cells. The effects on 2 STT cells was determined by stimulating the nerve while pinching the skin or skin and muscle of the receptive field; discharge rate decreased from an average of 28 spikes/s to 2 spikes/s during the stimulation period. In the other cell phrenic stimulation decreased spontaneous activity by 11 spikes/s. Two cells did not respond to phrenic stimulation. Phrenic afferent fibers were most likely activated because transection of the nerve proximal to the electrodes abolished the response. The cells were found in laminae I, IV, V and VII and received Aδ or Aδ and C-fiber input from cardiopulmonary afferents. In conclusion these results demonstrate that stimulation of afferent fibers in the phrenic nerve can modify the activity of STT cells. We speculate that afferent information arising from the diaphragm and subdiaphragmatic structures, such as the liver, might contribute information about visceral pain. (Supportd by NIH grants, HL22732, HL27260, HL00557, NS07114 and HL07340).

236.6 CORRELATION OF PRIMATE MEDULLARY DORSAL HORN NEURONAL RESPONSES WITH THERMAL DETECTION IN THE NOXIOUS RANGE. W. Maixner, D.R. Kenshalo, Jr., M.C. Bushnell, J.L. Oliveras and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

Previous studies from this laboratory (Bushnell et al., Soc. Neurosci. Abstr., 9:473, 1983) have demonstrated that both monkeys and humans are able to detect small temperature changes in the noxious range. In this study, we have correlated the activity of medullary dorsal horn wide-dynamic-range (WDR) and nociceptive specific (NS) neurons with the ability of behaving monkeys to detect small temperature increases in the noxious range.

Two rhesus monkeys were trained to perform a thermal detection task in the noxious range. Monkeys initiated a trial by depressing an illuminated panel button. Following panel press, a contact thermode positioned on the upper lip increased in temperature from a baseline of 38°C to a noxious (45°C to 48°C) temperature (T1). After a random time period of 3 to 9 sec the thermode increased an additional 0.2° to 1.0°C (T2). Monkeys received a fruit juice reward for releasing the panel button within 2 sec of the onset of T2. Single unit activity was recorded from 12 WDR and 10 NS medullary dorsal horn neurons during the behavioral task. In addition, median detection latency (MDL) to the onset of T2 was determined.

The MDL of the response to a 1°C shift (T2) was shorter the greater the preceding T1 (44°, 45°, 46°, 47° or 48°C). Similarly, the MDL was inversely related to the magnitude of peak neural discharge frequency produced by the 1°C shift for both WDR and NS neurons. In situations when T2 increased 0.2° to 1.0°C from a preceding T1 of either 45° or 46°C, MDL decreased as T2 increased. The MDL was also inversely related to the magnitude of peak neural discharge produced by a given T2 for both WDR and NS neurons. Equivalent MDL values for a T2 from a preceding T1 of either 45° or 46°C were associated with equivalent peak neural discharge rates for 3 WDR neurons.

These data demonstrate that the magnitude of discharge of WDR or NS neurons predicts detection latencies to small temperature increases in the noxious range. We conclude that both WDR and NS neurons are part of specialized central neural pathways capable of providing noxious thermal sensory-discriminative information.

236.7 THE ACTIVITY OF WIDE-DYNAMIC-RANGE NEURONS AND NOT NOCICEPTIVE SPECIFIC NEURONS PREDICTS THE DETECTION OF SMALL INCREMENTS IN NOXIOUS THERMAL STIMULI IN THE PRIMATE.
D.R. Kenshalo, Jr., W. Maixner, J.L. Oliveras, M.C. Bushnell and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

Monkeys can detect small increments in temperature that are superimposed on noxious thermal stimuli. We examined the responses of medullary dorsal horn neurons that might subserve the monkeys' ability to detect these small changes in temperature in the noxious range.

Two monkeys were trained to detect a second increase of 0.2^{o} to 1.0^{o}C (T2) after a temperature change from a 39^{o}C baseline to 45^{o} or 46^{o}C (T1) (See Maixner et al., this volume for complete details of psychophysical task). We recorded from 12 wide-dynamic-range (WDR) neurons and 10 nociceptive specific (NS) medullary dorsal horn neurons while the monkey was performing the psychophysical task. Two WDR and 4 NS neurons were antidromically activated from VPM of the thalamus.

The slopes of stimulus-response functions obtained during T1 and T2 were monotonic for both WDR and NS neurons. However, the slope of the stimulus-response function was greater for WDR neurons than for NS neurons for both T1 and T2. The animals' ability to detect near threshold increments in noxious temperature (0.2^{o} at 46^{o}C) was correlated with the discharge of WDR neurons. A correct detection by the monkey of T2 resulted in a statistically significant ($p < .05$) increase in discharge frequency of WDR neurons as compared to occasions when the monkey did not detect T2. In contrast, the response of NS neurons to T2 was not statistically related to the monkeys' detection of the stimulus.

These data suggest that WDR and NS neurons encode both large and small temperature changes in the noxious range. However, the increase in discharge frequency of only WDR neurons during T2 provides an accurate prediction of the monkeys' ability to detect near threshold changes in noxious thermal stimulation.

236.8 RECEPTIVE FIELD PROPERTIES OF SPINAL NEURONS PROJECTING TO THE ROSTRAL AND CAUDAL MIDBRAIN.
R.P. Yezierski and R.H. Schwartz*. Department of Anatomy, University of Mississippi Medical Center, Jackson, Mississippi 39216.

Recent anatomical studies using the anterograde transport of WGA-HRP have shown that there is a significant spinal projection to the rostral and caudal midbrain in the cat (Wiberg and Blomqvist, 1984; Yezierski, unpublished observations). Although it has been shown that the spinomesencephalic tract may constitute an important afferent pathway to supraspinal structures involved in nociception (Yezierski and Schwartz, 1984), the functional characteristics of neurons projecting to the rostral versus caudal midbrain have not been systematically evaluated. The present study was, therefore, designed to evaluate the response and receptive field (RF) properties of spinal neurons projecting to these midbrain regions.

Recordings were made from 55 identified spinomesencephalic tract cells in the lumbosacral spinal cord of adult cats. Cells were activated antidromically from stimulating electrodes positioned in the contralateral midbrain at thresholds ranging from 50-650 uA. Animals were anesthetized with alpha-chloralose and sodium pentobarbital (2 mg/kg/hr); expired CO_2 and core temperature were maintained within normal limits.

Cells projecting to the rostral midbrain were activated from the periaqueductal gray and adjacent reticular formation or from nucleus of Darkschewitsch. Cells projecting to these locations were divided into three categories: (a) high threshold (HT) with small excitatory RFs (9 cells); wide dynamic range (WDR) with excitatory RFs confined to the ipsilateral hindlimb (5); and (c) cells with no demonstrable RFs (15). Cells in the latter category typically had no background activity, while HT and WDR cells had moderate background activity which could be inhibited by noxious stimuli applied to the ipsi- and contralateral forelimbs, face or hindlimbs. Conduction velocities for cells projecting to the rostral midbrain ranged from 8-78 m/s. Spinal neurons projecting to the caudal midbrain responded to varying intensities of mechanical stimuli (12 cells), stimulation of deep tissue (4) or exclusively to high threshold stimuli (10). Seven cells projecting to the caudal midbrain had whole body excitatory RFs. Extensive inhibitory RFs were rarely observed for cells projecting to the caudal midbrain. Conduction velocities for caudally projecting cells ranged from 9-92 m/s.

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236.9 THE AXONAL BIFURCATION AND TERMINATION OF THE DOUBLY PROJECTING SPINAL DORSAL HORN NEURONS.
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Physiological and morphological evidence has been presented for the spinal dorsal horn neurons (SDHN) with branched axons ascending both the dorsal columns(DC) and dorsolateral funiculus(DLF). The present study is aimed at physiologically searching the branching and terminating sites of the neurons.

Laminectomies exposed spinal segments C1, C3-C6, and L6-S1 in the cat anesthetized with sodium pentobarbital and paralyzed with Flaxedil. Antidromic stimulation were delivered to the cervical (C4) DC and DLF that were dissected and electrically isolated apart from one another and apart from the rest of the spinal cord. Following identification of the neurons, high voltage stimulation of DLF at C1 was used to judge the termination of the neurons. A caudal dissection between DC and DLF was then made to find out the branching sites.

Twenty five neurons at depth corresponding to laminae III-IV of lumbosacral enlargement were antidromically driven from both the DC and the ipsilateral DLF. Antidromic spikes evoked from one of the funicular stimulation were found to disappear when the dissection between DC and DLF reached a point at segments C7-T4. No antidromic responses could be evoked by C1 stimulation even at stimulus intensity up to 100Volts. The thresholds of DC and DLF activation ranged from 1 to 30 Volts. The axonal conduction velocities in two funiculi were 34-65 m/sec. The differences in the conduction velocities between DC and DLF varied from 0 to 21 m/sec. Almost equal number of low threshold mechanoreceptive and wide dynamic range neurons were classified by receptive field stimulation. The diameters of afferent fibers to the neurons were found in the A-beta range.

The results suggest that ascending either DC or DLF, the SDHN's major axons seemed to give rise a branch at the upper thoracic and lower cervical segments of the spinal cord; in addition to project to the dorsal column nuclei, the SDHN's axons also terminated in the lateral cervical nucleus.

236.10 CORTICAL PROJECTION OF NOCICEPTIVE NEURONS IN THE PARALAMINAR ZONE OF THE CAT'S VENTROPOSTEROLATERAL NUCLEUS.
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Previous results have demonstrated that the ventral margin, or paralaminar zone, of the cat's ventroposterolateral nucleus (VPL) is differentiable from VPL proper in that it receives a moderately dense spinothalamic input and contains neurons responsive to both cutaneous and deep noxious stimulation (Craig and Burton, Neurosci. Abstr., 5:705, 1979; Honda et al., J. Neurophysiol., 49:662, 1983; Kniffki and Mizumura, J. Neurophysiol., 49:649, 1983). Similar nociceptive neurons have been recorded in area 3a of the cat's cortex (Iwamura et al., Pain, Suppl. 1:S213, 1981). We are testing the possibility that these areas may be related by using antidromic activation and retrograde labeling to examine the cortical projection of nociceptive neurons in the paralaminar VPL.

Up to now the most distinct results have been obtained for those paralaminar neurons responsive to noxious stimulation of the hindlimb that occur ventral to the forepaw representation in VPL proper. Such neurons can be antidromically-activated from the medial (hindlimb) pericruciate motor cortex, in stark contrast to the lateral sigmoidal (forepaw) sensory cortical projection of the dorsally adjacent cells in VPL proper. Similar differences have been observed for paralaminar neurons with forelimb receptive fields. Paralaminar nociceptive neurons have so far not been antidromically-activated from the lateral suprasylvian or anterior ectosylvian cortices. Retrograde labeling experiments with HRP and/or Fast Blue injections guided by antidromic recordings confirm that some paralaminar neurons ventral to the medial part of VPL project to medial pericruciate cortex.

These results provide further evidence differentiating the paralaminar zone of VPL, and indicate that its cortical projection maintains a separate, topographic order.

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237.1 CYTOCHROME OXIDASE STAINING OF ON AND OFF REGIONS IN THE RETINA, LGN AND STRIATE CORTEX OF TREE SHREW. T.T. Norton and M. Wong-Riley. Dept. of Physiological Optics, Univ. of Ala. in Birmingham, Birmingham, AL 35294 and Dept. of Anatomy, Med. Coll. of Wisconsin, Milwaukee, WI 53226.

Segregation of ON- and OFF-center channels has been reported in the LGN and striate cortex of tree shrew (Conway & Schiller '83; Norton, Kretz & Rager '83). In the LGN of monkey and ferret, where ON and OFF channel segregation has also been reported, the ON and OFF regions differ in the patterns of cytochrome oxidase (C.O.) staining (Kageyama & Wong-Riley '83). We examined whether a similar pattern occurs in the retina, LGN and striate cortex of 10 tree shrews which had been reacted histochemically for C.O.. In the retina, the outer and inner plexiform layers were highly reactive for C.O.. A clear zone separated the a and b inner plexiform sublaminae which seemed about equally reactive for C.O.. In the LGN, laminae 1 and 2, which contain mostly ON-center cells, were highly reactive for C.O.. Laminae 4 and 5, which contain mostly OFF-center cells, were also highly reactive for C.O.. LGN lamina 3 was distinctly paler than the other 4 laminae while lamina 6 seemed intermediate. These two laminae (3 and 6) receive input from small retinal ganglion cells, project to layer III of striate cortex and may be part of a W-like afferent pathway. In the striate cortex, layer IV was distinctly more reactive for C.O. than were the other cortical layers. However, this layer was not enzymatically homogeneous. Sublayer IVb, in which predominantly OFF responses have been found, was consistently more reactive than sublayer IVa, in which predominantly ON responses have been found. Approximately the middle 1/3 of layer IV stained more palely than either IVa or IVb. This pale region includes the cell-sparse cleft but extends considerably beyond the cleft into IVa and IVb. These results indicate that both the ON and OFF channels are quite active at the retinal, LGN and cortical levels in the normal tree shrew. In the cortex, the predominantly OFF subdivision (IVb) appears more reactive than the ON, while the middle third of layer IV has a metabolic level that is distinctly lower. The pattern at all 3 levels of the visual system may also reflect differences in the W, X and Y systems. An intriguing question raised by the cortical data is: why is IVa separated from IVb by a broad zone of low C.O. reactivity? Could this pale zone be produced by inputs from LGN lamina 3 which have eluded detection in pathway tracing experiments? Supported by NIH grants EY02909 and NS18121.

237.2 OCULAR DOMINANCE AND DISPARITY-SENSITIVITY IN CAT VISUAL CORTEX. Jill C. Gardner, Research Laboratory of Electronics, Massachusetts Institute of Technology, Rm. 36-864, Cambridge, Mass. 02139.

Ocular dominance "columns" or "bands" in the visual cortex have been demonstrated in a variety of species, but they have no known function. It has been widely accepted, however, that cells driven equally by the two eyes subserve binocular vision, while "monocular" cells subserve monocular vision. We report here on the relationship between ocular dominance (OD) and disparity-sensitivity in units of cat visual cortex.

Binocular interactions were examined in 250 units of cat area 18, and the 17/18 border. Stimuli were presented at different retinal disparities, and were moved in the same and in opposite directions on the two receptive fields. Indices of disparity-sensitivity, binocular inhibition and binocular facilitation were calculated for units of different ocular dominance groups.

A clear and consistent relationship was seen between ocular dominance and disparity-sensitivity, and this relationship was seen independently among units of the 17/18 border and units of area 18. Seventy-five percent of the units encountered showed disparity-specific binocular interactions, and these units were from all ocular dominance classes. Disparity-sensitivity varied systematically with ocular dominance, and contrary to what might have been expected, ocular unbalanced cells showed larger binocular interactions than ocular balanced cells. Units which could be driven only by one eye (OD groups 1 and 7) were most sensitive to retinal disparity, while units driven equally by the two eyes (OD group 4) were the least sensitive to disparity. Overall, units dominated by the ipsilateral eye (OD groups 5,6 and 7) showed larger binocular interactions than units dominated by the contralateral eye (OD groups 1,2 and 3). Larger binocular interactions in units were due to both stronger binocular inhibition and stronger binocular facilitation.

The results of the present experiment indicate that units of all ocular dominance classes, and particularly the "monocular" cells of OD groups 1 and 7, play a role in binocular vision. The strong relationship seen between ocular dominance and disparity-sensitivity, suggests that common mechanisms underly these different responses. The results indicate that the ocular dominance columns of cat visual cortex function in the organization of disparity-sensitive neuronal systems.

237.3 CONTRAST COLUMNS IN THE STRIATE CORTEX OF THE MINK. S. LeVay and S.K. McConnell, Salk Institute, La Jolla, CA 92137.

On- and off-center relay neurons occupy separate laminae in the mink's LGN, and their axons terminate in alternating, partially overlapping patches in layer 4 of the striate cortex (PNAS 81:1590;1984). We have now studied the response properties of neurons in this area, with special attention to their contrast preference. Most properties were studied qualitatively, but histograms were prepared of averaged on- and off-responses to stationary, optimally-oriented slits as a function of position across the receptive field.

The types of visual responses obtained in each layer were generally similar to those described in the cat. In layer 4, simple cells, E-on, E-off, and a few complex cells were recorded. Most simple cells had only two excitatory subfields, one on and one off. These could be about equal in strength, or one subfield could be considerably more powerful than the other (on- or off-dominated simple cells). At the extremes of this range were the E-on and E-off cells: these possessed only one excitatory region, though responses were generally suppressed by enlargement of the stimulus into flanking zones. Layer 4 cells often showed a preference for light or dark moving edges in a manner predictable from their stationary responses. Most cells responded to both light and dark moving slits, but a few responded selectively to slits of the same sign of contrast as the preferred stationary stimulus. In layer 6, both simple and complex cells were found. In layers 2,3 and 5 most cells had complex fields. Tested with stationary slits, they lacked subfields, but there was a spectrum of response types from exclusively on, through equally balanced, to off. Compared with simple cells, there was a relative predominance of equally balanced units.

In penetrations perpendicular to the layers, successive units showed similar contrast preference (on-dominated, balanced, or off-dominated), while on tangential penetrations contrast preference changed at irregular intervals. The grouping was most obvious in layers 4 and 6, but it was also detectable in other layers. On occasions when we were able to record geniculate afferent activity simultaneously with cortical units, the contrast preference of the two was similar. Orientation and ocular dominance columns were also present.

We conclude that the anatomical sorting of the on- and off-center afferents in the mink's cortex gives rise to a functional columnar system for contrast preference, analogous to that for ocular dominance. It remains to be determined whether contrast columns are a peculiarity of the mink's visual system. (Supported by NIH EY0551 and NSF SPI81-66337.)

237.4 ABOLITION OF CORTICAL DIRECTIONAL SELECTIVITY IMPAIRS DIRECTION DISCRIMINATION OF CATS. Tatiana Pasternak, Robert A. Schumer, Martin S. Gizzi and J. Anthony Movshon. Center for Visual Science and Center for Brain Research, University of Rochester and Department of Psychology, New York University.

We raised 7 cats in an environment illuminated at 8 Hz by a 3 μsec strobe flash. At the age of 8 months, they were moved to a normally lit environment, and subjected to behavioral testing. We compared the ability of four of these cats to detect moving sinusoidal gratings with their ability to discriminate the direction of the gratings' motion. Control data were obtained from 3 normally-reared animals. In the detection experiment the cats were presented with a vertical grating and a uniform field of the same mean luminance and were rewarded for selecting the grating. In the discrimination experiment they viewed two identical vertical gratings moving in opposite directions and were rewarded for selecting the rightward moving grating. We measured contrast sensitivity using this paradigm for 0.28 c/deg gratings moving at 1.1, 2.2, 4.4 and 8.8 Hz (4, 8, 16 and 32 deg/sec).

Normal cats showed equal sensitivity in the detection and direction discrimination tasks. The contrast sensitivity of strobe-reared cats for detecting the moving gratings was within the normal range. Their contrast sensitivity for discriminating the direction of motion was, however, markedly reduced at all stimulus speeds.

At the conclusion of the behavioral testing, we recorded from 173 units in the striate cortex of three such animals. Like Cynader and Chernenko (1976), we found that 8-Hz stroboscopic rearing utterly abolished directional selectivity in this neural population, while leaving such other fundamental receptive field characteristics as contrast sensitivity and orientational, spatial and temporal selectivity largely unaltered. Furthermore, the units in strobe-reared cats trained and tested behaviorally for some months were indistinguishable from those recorded from other cats strobe-reared until the day of recording.

Our results demonstrate that the detection of moving gratings does not require the presence of cortical directional selectivity. In the absence of directional neurons, however, the discrimination of direction of motion is grossly impaired. Indeed, given that only 5% of the cortical neurons in these cats showed significant directional assymetry in their responses, the ability of the cats to discriminate direction at suprathreshold contrasts was remarkable. (NIH grants: EY04118,EY01319,EY02017,EY00187; NSF grant: BNS582-16950.)

237.5 FUNCTIONAL TOPOGRAPHY IN AREAS 17 AND 18 OF CAT VISUAL CORTEX. M. Cynader, J.A. Matsubara, N.V. Swindale. Depts of Psychology and Physiology, Dalhousie Univ. Halifax, N.S. CANADA B3H 4J1.

We have examined physiological response properties across the cortical surface in cat visual cortex. Recording sites were spaced approximately 300 μm apart in a grid-like pattern on the cortical surface. The depth of the recordings was about 400 μm. At each recording site the following properties were studied: 1) receptive field location, 2) receptive field size, 3) ocular dominance, 4) orientation, 5) direction and 6) velocity selectivity.

Areas 17 and 18 differed in a variety of ways. Receptive field area, for comparable eccentricities, was about four times larger in area 18 than in 17. Preferred velocities of area 18 cells were higher than those of 17. The retinotopic map was markedly anisotropic in 18, with the magnification factor for vertical at least twice that for horizontal visual space. No comparable topographic anisotropy was observed in area 17.

In both areas, neurons with similar functional properties occured in patches. The fine grain maps revealed clusters of cells dominated by one or the other eye. Cells with similar velocity preferences were also clustered together as were cells with similar orientation selectivity. However, the cortical surface representations of different response features varied in their orderliness, periodicity, interanimal consistency and direction of elongation. The most regularly-arranged feature observed in these experiments was that for preferred orientation. Units preferring the same orientation were laid out in branching bands running roughly medial-lateral (area 18) or dorsal-ventral (area 17). The period of these bands measured by spectral analysis was roughly 1.25mm.

Studying the surface distribution of neuronal properties makes it clear that it is not always possible to provide full coverage of each point in the visual field by neurons responsive to all combinations of stimulus preferred orientation, ocularity and velocity. The consequences of these incomplete representations will be considered.

237.6 PHYSIOLOGICAL CORRELATES OF THE ANATOMICAL CONNECTIONS WITHIN AND BETWEEN AREAS 17 AND 18 OF CAT VISUAL CORTEX: J.A. Matsubara, M. Cynader, N.V. Swindale. Depts of Psychology and Physiology, Dalhousie Univ. Halifax, N.S. CANADA B3H 4J1.

We previously reported on the physiological rules governing the local connections within area 18. Here we consider the physiological correlates of the intrinsic connections within area 17 and the extrinsic connections between areas 17 and 18.

Our methods consist of mapping portions of both areas 17 and 18 in the same hemisphere. Injections of WGA-HRP and succinylated-Concanavalin A are placed into physiologically-identified points in our maps. Both tracers travel antero- as well as retro-grade and both can be visualized in the same section of tissue.

The intracortical and intercortical connections arising from an injection site differ as follows:

1. Whereas the local, intrinsic connections within a given area are typically punctate in their distribution the extrinsic connections form bands over the cortical surface. The bands run roughly perpendicular to the 17/18 border.
2. A spherical injection in area 17 produces labelling in area 18 which extends further in the anterior-posterior than the medial-lateral direction. In contrast a spherical injection in area 18 produces labelling in area 17 which spreads further in the dorsal-ventral than the anterior-posterior direction. This is consistant with a reciprocal projection between corresponding retinal points in two areas, one of which contains an anisotropic map of visual space.
3. The local connections within a given area are strictly reciprocal: the areas which contain labelled cell bodies also contain the granular reaction product typical of anterograde and/or collateral transport. However, the extrinsic connections often exhibit areas of pure anterograde and/or collateral transport.
4. Neurons associated with the local connections are distributed in a columnar fashion whereas the neurons associated with the intercortical connections are not.

We thank Dr. D.W. Nance for assistance with immunocytochemical techniques.

237.7 QUANTITATIVE ANALYSIS OF CAT SIMPLE RECEPTIVE FIELDS IN TWO SPATIAL AND SPATIAL FREQUENCY DIMENSIONS. L. Palmer and J. Jones.* University of Pennsylvania, Philadelphia, Pa. 19104

Recent theoretical work with simple cells has emphasized the importance of the 2-dimensionality of the receptive field (RF) in both space (S) and spatial frequency (SF) domains. To date, 2-dimensional properties of the simple RF have only been inferred. We describe here general techniques for obtaining full 2D characterizations and draw some important conclusions from the data so obtained in this and the accompanying abstract.

A computer controlled CRT image generator (Innisfree) was used in both domains. In the S domain, a 16x16 grid was centered on the RF using a computer-assisted handplotting procedure. Small bright and dark stimuli were presented singly in random order at each of the 256 net points. Stimulus duration was typically 25 to 100 msecs. Cross correlation was performed on-line between the spike train and the stimulus sequence. The difference between the two correlations (bright - dark) was interpreted as the 2D spatial impulse response of the RF. In the SF domain, another 16x16 grid was specified centered on the responsive region of the cell. Drifting sinusoidal, non-saturating grating stimuli were randomly presented at each SF-orientation coordinate in the grid. Cyclegrams were generated for the last 4 of 5 cycles at each grid point in each pass. From the 256 cyclegrams response amplitude at selected harmonics was plotted as a 16x16 surface.

In the S domain, simple RFs were found to consist of 2 to 4 elongated, parallel regions excited alternately to bright or dark. The most responsive regions were always near the RF center and the response diminished smoothly in both the width and length dimensions. In the SF domain, responsive areas were circular or elliptical. Responses to changing orientation were symmetrical about the optimal at all SFs. Peak sensitivity to SF was often skewed towards the high or low end of the cell's range; this was true at all orientations. In direction selective cells the two peaks were symmetrically placed and had exactly the same shape. Supported by EY-04638.

237.8 SIMPLE RECEPTIVE FIELDS IN CAT STRIATE CORTEX: A COMPARISON WITH GABOR FUNCTIONS IN TWO DIMENSIONS OF SPACE AND TWO DIMENSIONS OF SPATIAL FREQUENCY. J. Jones* and L. Palmer (Spon: J. Saunders) University of Pennsylvania, Phila., Pa. 19104

Recently, several authors have advanced the hypothesis that simple receptive fields in striate cortex can be modelled as Gabor functions. The Gabor function is interesting in this context because it has been shown to provide an 'optimal tradeoff' between simultaneous localization of a stimulus in space and spatial frequency. The Gabor function can be generalized to two dimensions (2D) while preserving its optimal properties. This generalization is of obvious interest to the study of simple receptive fields.

We have developed methods for measuring the 2D response properties of visual receptive fields in space and spatial frequency. We have used these methods to examine the 2D response surfaces of 25 simple cells in cat striate cortex. Here we compare the observed response surfaces with those predicted by the 2D generalization of the Gabor function.

In the space domain, we find that every simple receptive field is well-fit by a 2D Gabor function. Although we have not yet performed a statistical analysis of this result, the fit is so robust that the comparison can be made by eye.

We also compared the observed spatial frequency tuning surfaces with the 2D fourier transform of the space domain data. We find that in some cases, the predicted tuning surfaces agree well with the observed data. In the remainder of the cases, observed surfaces agree with predicted surfaces in one direction (parallel to the spatial frequency axis at optimal orientation) but observed bandwidths are narrower than predicted in the orthogonal direction. This suggests non-linear spatial effects in simple cells along the 'length' axis, but linear spatial summation along the 'width' axis. We conclude that 2D Gabor functions provide a good model for simple cell receptive fields in 2D space, but the assumption of spatial linearity does not account for all of the data in 2D spatial frequency. Supported by EY-04638.

237.9 RED, BLUE, GREEN, YELLOW AND NONCOLOR ZONES IN FOVEAL STRIATE CORTEX OF THE AWAKE MONKEY. B.M. Dow and R.G. Vautin*, Neurobiology Division, Physiology Department, School of Medicine, University at Buffalo, SUNY, Buffalo, NY 14226.

Color tuning curves have been obtained from 218 cells in foveal striate cortex of alert, behaving monkeys[1]. The present report is based on a subset of 171 cells recorded in 43 systematic vertical or nearly vertical penetrations (3-7 cells per penetration).

Two types of penetrations through zones with nonoriented upper layer cells (presumed cytochrome oxidase puffs[2]) are found to occur in approximately equal numbers. Type R (red) penetrations contain mostly long-wavelength-sensitive cells in the upper layers; Type B (blue) penetrations contain mostly short-wavelength-sensitive cells in the upper layers. Types R plus B constitute approximately 1/3 of foveal striate penetrations, which is consistent with measurements of the ratio of puff size to total cortical surface area in the foveal region[2].

Color tuning in both Type R and Type B penetrations is maximal in the middle layers (microelectrode depth range: 0.6-1.4 mm), rather than in the upper layers where the puffs are located. As a recording electrode descends, the tuning curves become progressively narrower and the peaks move toward one or the other end of the spectrum. Typically at microdrive depths of 0.8-1.0 mm one begins to encounter secondary tuning curve peaks at the opposite end of the spectrum from that of the more superficially located cells. Some cells in this transitional zone, which may correspond to layer 4B, characteristically respond to stimuli at either end of the spectrum, but fail to respond to midspectral stimuli (endspectral double peak cells). Below the transitional zone one sometimes encounters cells with a single response peak at the opposite end of the spectrum from that of the upper layer cells.

Penetrations with oriented cells in the upper layers (presumed interpuff penetrations) contain mostly cells that respond well to midspectral stimuli. There are 3 classes of such penetrations: Type G (green), Type Y (yellow), and noncolor.

The orderliness with which color is organized in foveal striate cortex is comparable to what has been described previously for orientation and ocular dominance.

Supported by NIH grants EY02349 and T32 EY07019).

References:
1. Vautin and Dow, Neurosc. Abs. 9: 822, 1983.
2. Livingstone and Hubel, J. Neurosc. 4: 309-356, 1984.

237.10 **Interhemispheric connections of area 17 in adult tree shrews (Tupaia belangeri).** G. Rager, M. Pritzel* and R. Kretz*, Institute of Anatomy, 1, rue Gockel, CH-1700 Fribourg, Switzerland

The callosal projection of the primary visual cortex in cats and rhesus monkeys is confined to the vertical meridian along the area 17/18 border. This is true for the adult animal only. In the newborn, however, a much larger part of area 17 is connected to the contralateral side. During development this projection becomes confined to the narrow stripe observed in the adult animal. Since the tree shrew differs from cats and rhesus monkeys in that the two eyes are represented not in alternating columns but in sublaminae, one could expect that callosal projections may be different, too. Therefore, we have investigated the interhemispheric projections of area 17 in this animal.

The visual cortex of anesthetized animals was injected at selected stereotactic coordinates using micropipettes (inner tip diameter approx. 30 μ). Pipettes were filled with 30% HRP dissolved in 0.01 M NaCl. At depths of 500, 1000 and 1500 μ HRP was ejected either iontophoretically or by pressure. After a survival time variing between 20 to 51 hours, brains were cut on a frigotome and processed using TMB as chromogen.

The following results were obtained. Along the 17/18 border we found a heavy projection to the corresponding site in the contralateral hemisphere with a preponderance of the representation of the central retinal area. This projection along the vertical meridian can be called homotopical. A systematic analysis of the remaining binocular segment of area 17, accessible to penetrations, showed that it is also interconnected with the contralateral hemisphere. However, neurons projecting to a given injection site are no longer confined to the homotopical place but can also be widely dispersed, thus, they are also heterotopical. In addition, the heterotopic projection seems to be less dense than the homotopic one.

As these data show, the callosal projections do not become restricted to the 17/18 border during development. Thus, the tree shrew is different from cats and monkeys not only by the fact that its binocular representation in the visual cortex is segregated in laminae rather than in columns, it also maintains its callosal connections, at least to a certain extent, during ontogenesis.

CONTROL OF POSTURE AND MOVEMENT II

238.1 RECONSTRUCTION OF THREE DIMENSIONAL ORGANIZATION OF MUSCLES AT THE HIP JOINT USING COMPUTED TOMOGRAPHY, P.Dev, S.T. Woolson*, and L.L.Fellingham*, Contour Medical Systems, Mountain View, CA 94043.

A biomechanical model for posture control requires knowledge of joint geometry, muscle force and moment arms. A non-invasive method of measuring these parameters allows the model to be more rigorous, take into account individual differences and obtain measurements from human subjects. A method is presented of using a computed tomography (CT) scan to obtain a description of the three-dimensional (3D) organization of muscles around the hip joint.

CT data from scans of cadavers and patients are transferred to a system which is composed of a 32 bit microprocessor, a high resolution color monitor, a large disk for storage and a tablet for rapid interaction with the system. Each scan slice is automatically analyzed to select out muscle tissue and an algorithm rapidly defines the surface of muscle. The contours from each slice are stacked in the computer, rotated to any angle of view and displayed on the monitor as a shaded solid (3D display).

Muscle groups and some individual muscles are clearly distinguished in the 3D display. Through graphics editing, individual muscles are separated out for rotation and viewing. The muscle moment arm is measured. Muscle cross-sectional area is obtained as a measure of muscle peak force.

This method for muscle contouring, display and measurement of moment arm and cross-sectional area has been tested on cadavers and patients with congenital hip dysplasia. The abductor muscles, gluteus maximus, medius and minimus and the adductor, iliacus, are clearly distinguished and are separable. Muscle atrophy on the affected side is clearly seen and is measurable.

Measurements of force and moment arms in patients are used in a simple force balance model to show the difference in equilibrium position of the normal and affected legs.

238.2 PROCESSES UNDERLYING THE INITIATION AND SPECIFICATION OF RAPID ISOMETRIC PULSES IN A HUMAN TRACKING TASK. W. Hening and C. Ghez, Dept. of Neurology and Center for Neurobiology and Behavior, Columbia-Presbyterian Med. Ctr., NY, NY 10032.

When subjects (Ss) aim rapid force pulses at targets of varied amplitudes (choice condition), their responses are accurately scaled if Ss can respond "when ready." However, if Ss respond as soon as possible (RT constraint), the range of response amplitudes is constricted. This constriction is not seen in blocks of constant target size (simple condition) (Hening, Vicario, and Ghez, 1983). These findings suggest that, in a choice RT condition, responses are initiated before their desired amplitudes are completely specified. The present experiments were performed to uncover the task features that determine the range of choice responses and to establish the time course of response specification.

Four normal adult Ss produced rapidly rising isometric force pulses (finger or elbow flexions) to match target shifts displayed on an oscilloscope. In experiment 1, Ss responded under an RT constraint while presented with blocks of trials in which either the range of target forces or their probabilities was varied. We found that the constricted range of choice responses results from a "central tendency bias" dependent upon the frequency distribution of target amplitudes. In experiment 2, Ss were taught to initiate responses in synchrony with the last of a series of 3 clicks. Target shifts were presented for intervals of 50 to 500 msec (S-R interval) before the synchronizing click, allowing us to control S-R interval and to dissociate the process of response initiation from that of response scaling. For long S-R intervals (>400 msec), choice responses were accurately scaled. However, as S-R interval was shortened, the range of response amplitudes became increasingly constricted. For intervals equal to previously observed RTs (150-300 msec), constriction of range was similar. For brief S-R intervals (<100 msec), responses to all target amplitudes were clustered near the center of the target force range. Based on these results, we propose that the response to a target stimulus initially derives from a precomputed estimate of the required response which, following target presentation, is gradually replaced by a more accurate representation. Thus, response specification is a continuous or iterative process evolving over an interval that greatly exceeds the minimum reaction time and response initiation does not depend upon completion of this process.

Supported by the Dystonia Medical Research Foundation and NS19205.

238.3 DOES PROPRIOCEPTIVE INPUT INFLUENCE ACCURACY OF ONGOING VOLUNTARY FORCE RESPONSES? P.J. Cordo. Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, OR 97209.

During isometric force tracking by human subjects, a short latency error correction is produced about 100 ms after response onset which has been shown to be unrelated to visual feedback. Several mechanisms might be responsible for error detection and this corrective action, one of which is closed-loop proprioceptive feedback control. Previous studies examining the role of proprioceptive feedback in producing accurate voluntary movement have employed various techniques to eliminate possible proprioceptive influences on movement accuracy: ischemic nerve block, tendon vibration and use of patients with dorsal column lesions or pan-sensory neuropathy. However, each of these techniques has particular problems which make their results equivocal. In the present study, proprioceptive control was examined by creating an error which could be detected only by proprioceptive feedback and determining whether appropriate corrective action was taken.

Seated human subjects performed isometric force tracking responses with the elbow musculature to a visual step stimulus. When stimulus amplitude was unpredictable, subjects' initial response was stereotyped and uncorrelated with stimulus amplitude and this phase followed by a visually unrelated force correction. In order to determine what, if any, proprioceptive control was involved in this correction, the effect of small unexpected movements of the manipulandum were examined. These handle movements were designed to produce slightly larger force errors prior to the correction compared to totally isometric responses. Response errors at the beginning and end of this force adjustment were compared for trials with and without mechanical perturbations to see if any of the additional error produced by the perturbation was corrected. In trials with handle movements, the additional error induced at the onset of this correction was still present after the correction. Furthermore, the absolute amount of error corrected in either set of trials (with or without perturbation) was the same. These results indicate that this correction is not based on sensory feedback relating to the initial response and, therefore, must be produced by some form of open-loop or efference copy mechanism.

238.4 ACCURACY OF PLANAR ARM MOVEMENTS. T. Zeffiro and D. Claman*. Neurology Service, Mass. Gen. Hosp., Boston, MA 02114 and Dept. Psychology, MIT, Cambridge, MA 02139.

In order to explore the spatial characteristics of human movement, we investigated errors made in achieving final position while subjects performed arm movements without visual guidance.

Five right-handed subjects performed a step-tracking task in darkness while comfortably restrained in front of a 20" x 20" digitizing tablet. Beneath the translucent surface of the tablet were mounted arrays of LEDs that were invisible until activated. The subject, holding a stylus in one or the other hand, was instructed to move to a starting position that would appear in various locations on the tablet's surface. Once the proper starting position was attained, a trial would be initiated by the application of light pressure to the stylus tip. This would result in the immediate appearance of the target LED, which was extinguished as the movement commenced, leaving the subject in total darkness. Subjects were instructed to move as quickly and accurately as possible without making corrective movements. No feedback as to the magnitude of the final position error was provided. Six different start and 36 target locations were studied; combinations thereof were presented in pseudorandom order. Each subject performed a total of 1748 movements, alternating hands after each 108 trials. Final hand position was sampled and stored for later analysis.

For each movement, we defined the constant error vector (CEV) as the vector originating at the target position and terminating at the final hand position. Statistical analysis of the variation of the CEV with hand, starting location, target location, movement amplitude, and movement direction yields the following observations: (1) the major component of the CEV tends to be aligned with the movement direction; (2) the magnitude of the CEV is linearly related to the movement amplitude except at the farthest targets; (3) the vast majority of errors are overshoots; (4) CEV amplitude is uniform across target locations; and (5) CEV amplitude is independent of the hand used.

We conclude that the constant errors attending planar arm movements are strongly dependent upon movement amplitude and direction and relatively unrelated to absolute location in the task space. The observed absence of hand effects suggests that the control of this class of arm movements is not lateralized.

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238.5 COORDINATION BEFORE HEEL-OFF IN SQUAT JUMPS BY HUMANS IS CRITICAL TO THE ACHIEVEMENT OF MAXIMAL HEIGHT. F.E.Zajac[1,2], W.S. Levine[3]*, Y.M. Cho[3]* and M.R. Zomlefer[2]. Mech. Eng. Dept.,[2] Stanford Univ., Stanford, CA 94305[1], Palo Alto VA Med. Ctr. and Elect. Eng. Dept., Univ. of Maryland[3].

Based on our past theoretical and experimental studies of maximal height jumps by humans and cats (Levine et al., IEEE Trans., Vol. AC-28:1008-1016, 1983), we have hypothesized that once the heels leave the ground muscles should be either fully activated or inactivated until the rest of the body lifts off. However, on-off coordination of muscles is not needed prior to heel-off. The event separating the first part of propulsion from the second part is the biomechanical and muscle state at heel-off. To understand better the role of the heel-off state in integrating these two parts of propulsion, we studied jumps that started from a squat, in one case with feet flat on the ground and in the other with toes contacting the force-plate. A Selspot system was used to detect leg joint trajectories and trunk motion. Ankle, knee and hip torques were estimated by solving the inverse dynamics problem. EMG activity was recorded intramuscularly from knee and ankle flexor and extensor muscles. Complementary theoretical data was obtained from a computer model of the heel-off to lift-off phase of these jumps. The 4-segment model included skeletal dynamics and torque dynamics at the ankle, knee and hip. Torque dynamics were estimated from isometric and isovelocity records obtained from MVC by the same subjects. We found that the kinematics obtained from the solution to the optimal control problem are similar to the observed kinematics, since joint angles are not sensitive to changes in the control. Torques generated by our subjects were, however, noticeably different from torques computed from the model. We believe that series elasticity in tendon and muscle must be accounted for before these differences can possibly be resolved. Also, a reduction by 50% in knee strength in the model produces, qualitatively, the same analytical result. The reason is that the torque at heel-off is still achievable despite the halved knee strength and muscle dynamics are slow relative to duration. Strength does limit, somewhat, the achievable heel-off state. The point is, performance is more sensitive to heel-off state than it is to strength per se. Our results suggest that the crucial feature of the jump is the attainment of the optimal heel-off state, which requires neither maximal activation of muscles nor unique muscular coordination. Supported by NIH grant NS17662 and the Veterans Administration.

238.6 SYNCHRONIZATION OF MOTOR UNITS WITHIN DIFFERENT MUSCLES. C.J. De Luca, H. Broman and B. Mambrito. NeuroMuscular Research Laboratory, Children's Hospital Boston, MA 02115, and Liberty Mutual Research Center, Hopkinton, Ma 01748.

Synchronization of motoneuron or motor unit discharges may be defined as their tendency to discharge at or nearly at the same time. This phenomenon has been the object of fascination for many investigators since 1907 when Piper first described the "rhythm" in the myoelectric signal. Although, motor units have been reported to "synchronize" during strong and fatiguing contractions, the evidence is weak due to the technical complexity of obtaining correct data. The myoelectric signal decomposition technique which we have described at previous meetings of this society enables us to obtain accurate measurements of the inter-pulse intervals of motor unit discharges throughout the full range of muscle contraction levels.

Experiments were performed on 12 normal subjects. They were required to maintain an isometric contraction at either 30% or 60% MVC for at least 20 seconds. A total of 102 motor unit action potential trains from 30 contractions were analyzed. The deltoid and first dorsal interosseous muscles were studied. The conditional intensity function (similar to the cross-correlation function) was calculated and used to measure the amount of synchronization among the motor units. Each contraction showed evidence of synchronization which consisted of discharges that occured within 3 ms. No statistically significant difference was noted in the amount of synchronization at the two force levels, but the first dorsal interosseous displayed approximately twice as much synchronous activity than the deltoid at both force levels. (Each comparison was based on 2,000 to 5,000 motor unit discharges.)

In one subject it was possible to obtain correct data from four motor units in the tibialis anterior during 50% MVC isometric contraction lasting 144 s. In this case it was noted that the amount of synchronization did not increase with time, but instead it was most often present at times when the force output demonstrated sharp perturbations. Synchronization tended to decrease when a new motor unit was recruited. This latter observation is consistent with the obvservation that the deltoid muscle, which uses recruitment as the prime mechanisms for augmenting the force output, displayed less synchronization than the first dorsal interosseous.

The data supports the notion that some (possibly most) of the synchrony is induced by the stretch reflex loop.

(This work was supported by Liberty Mutual Ins. Co.)

238.7 GROUP II MUSCLE AFFERENTS AND NOCICEPTIVE CUTANEOUS AFFERENTS INTERACTING IN SEGMENTAL REFLEX PATHWAYS IN THE CAT. E.D. Schomburg*, H. Steffens*, and T. Behrends* (SPON: U. Kuhnt). Physiologisches Institut der Universität, D-3400 Göttingen, West Germany.

Group II afferents may converge onto common interneurones in reflex pathways used by afferents from low threshold mechanoreceptors (Behrends, T., Schomburg, E.D., and Steffens, H., Brain Res., 265: 125, 1983) or group III and IV muscle afferents, which originate from ergoceptors or nociceptors (Kniffki, K.-D., Schomburg, E.D., and Steffens, H., Brain Res., 218: 342, 1981). This rose the question whether group II muscle afferents may interact with assured nociceptive afferents in segmental reflex pathways to α-motoneurones.

In anaemically decapitate high spinal cats lumbar α-motoneurones were intracellularly recorded and the spatial interaction between responses from electrically activated group II muscle afferents and afferents from thermically (radiant heat graded from 40°C-60°C) activated cutaneous nociceptors of the hindpaw was investigated.

EPSPs in flexor and extensor motoneurones as well as IPSPs in extensor motoneurones evoked by group II muscle afferents were distinctly facilitated by nociceptive afferents activated by radiant heat of more than 43°C to 45°C (measured at the skin surface). The afferent nociceptive inflow partly induced a change of the membrane potential of the motoneurones. The facilitation of the group II EPSPs did not correlate to such changes of the membrane potential. For increased IPSPs a facilitation was only assumed, if the membrane potential was not changed or slightly hyperpolarized. The observed facilitation of the PSPs was not accompanied by comparable changes in membrane resistance.

The results reveal that group II muscle afferents may not only project onto common segmental reflex pathways with non-nociceptive but also with nociceptive afferents. This confirms the important role group II muscle afferents may play in the multisensorial control of movements (cf. Lundberg, A., Progr. Brain Res., 50: 11, 1979).

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238.8 EARLY AND LATE CONDITIONED REFLEXES DURING DEVELOPMENT IN THE CAT. P. Bawa, Department of Kinesiology, Simon Fraser University, Burnaby, B.C. V5A 1S6

In adult cats it has been shown (Eccles and Rall, 1951) that after the application of a conditioning stimulus to muscle afferents, the test pulse reveals an initial reflex activity (TR1), presumably monosynaptic, at test intervals of about 10 msec and no reflex response for test intervals of 15 to 60 msec. A second phase of reflex activity (TR2) appears for test intervals longer than 50-60 msec. The behaviour of the conditioning reflex (CR), TR1 and TR2 were investigated in young kittens in order to study the nature of time course of development of the monosynaptic reflex.

MG and LGS muscle nerves were stimulated in nembutal anaesthetised kittens of different ages. Reflex responses were recorded from proximal ends of cut L7 or S_1 ventral roots. The conditioning-testing stimulus interval was varied from 1 msec to 2 seconds.

For all ages, the amplitude of TR1 (when present) was inversely related to the amplitude of CR, while the amplitude of TR2 was directly related to the amplitude of CR. In kittens younger than 2 weeks of age no TR1 was ever observed, and TR2 could never be elicited before a test interval of 90 msec. In kittens between 2-4 weeks of age, TR1 was very small and peaked between 1-2 msec following CR; TR2 was seen for intervals of 60-70 msec. In kittens older than four weeks, a maximal TR1 was observed for test intervals of 6-10 msec, and TR2 was observable for test intervals as short as 40 msec.

These observations on monosynaptic reflexes may be interpreted to reflect the properties of the appropriate motoneuron pools. The behaviour of TR1 suggests that the amount and time course of temporal summation in motoneurons of young kittens is very different from that in adult cats. The nature of TR2 suggests extremely long after hyperpolarisations in motoneurons of young kittens which approach more adult-like values in kittens older than 4 weeks. The contribution of recurrent inhibition to the behavour of TR1 and TR2 must also be considered when interpreting the varying nature of these test responses.

This work was supported by NSERC.

238.9 STRETCH REFLEX BEHAVIOR OF A LIMB WHEN MORE THAN ONE LIMB SEGMENT IS FREE TO MOVE. J. F. Soechting and F. Lacquaniti*. Laboratory of Neurophysiology, University of Minnesota, Minneapolis, MN 55455.

The behavior of the stretch reflex has been customarily studied by applying force perturbations (such as torque pulses) to a given limb segment and recording electromyographic activity evoked by the perturbation. Motion of other limb segments is usually prevented by means of suitable restraints. In general, however, a perturbation applied to one limb segment will result in angular motion of all segements of that limb. Little is known about the behavior of the stretch reflex in this condition. The question is of interest because it may more closely approximate behavioral situations and also, because different functional interpretations of the stretch reflex lead to qualitatively different predictions regarding reflex behavior.

For example, a force applied to the upper arm tending to produce backward extension at the shoulder will also result in flexion of the forearm by virtue of the dynamic linkage between the two limb segments. If the stretch reflex acts as a length servo, one should expect reflex activation of elbow extensors, which lengthen, and inactivation of elbow flexors, which shorten. Instead, if the reflex acts to counteract changes in torque at the elbow joint, one would expect activation of elbow flexors to counteract the elastic forces due to the stretching of elbow extensors. At the shoulder, both hypotheses would predict activation of shoulder flexors. Experiments were done on human subjects to test for these hypotheses. Pulses of force were applied to the forearm or the upper arm, angular motion of the two limb segments was measured and electromyographic activity of biceps, triceps and anterior deltoid was recorded. It was observed experimentally that reflex activity in elbow flexors and extensors was always in opposition to the changes in elbow torque evoked by the perturbation but not consistently in opposition to the changes in muscle length. The data are thus qualitatively consistent with the hypothesis that the stretch reflex acts to counteract changes in net torque at a joint. Studies are in progress to test this hypothesis quantitatively.

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238.10 REFLEX EFFECTS OF AN INSECT PROPRIOCEPTOR CHANGE DURING PERIODS OF ACTIVE SEARCHING MOVEMENTS. Sasha N. Zill, Dept. Anatomy, Univ. Colo. Med. Sch., Denver, CO 80262.

Reflex effects of many types of proprioceptive sense organs have been shown to change during active movement (Vedel, J. P., J. Exp. Biol., 101:121, 1983) or walking (Forssberg, H., et al., Brain Res., 85:103, 1975), but few previous studies have examined this plasticity of reflexes at a cellular level. I have examined the reflex effects of a joint angle receptor of the locust hindleg, the metathoracic femoral chordotonal organ, through intracellular recordings from identified motoneurons to leg muscles and have found that these reflexes systematically change in sign and mode of action during periods of active searching movements.

Locusts (Schistocerca gregaria) were restrained in wax with one hindleg free to move and the metathoracic ganglion exposed for intracellular recording from motoneurons to tibial muscles. Step displacements were applied to the main ligament of the chordotonal organ that mimicked 10 - 15° changes in the angle of the femoro-tibial joint. The tibial segment of the leg was initially allowed to rest against a support during a recording then released to induce active searching movements.

Tibial motoneurons showed resistance reflex responses opposing apparent joint movements when the tibia was resting against a support. These responses consisted of constant, short latency (18 - 22 msec.) phasic excitatory post-synaptic potentials that were followed by periods of tonic excitation of variable magnitude and duration. These resistance reflexes could function in load compensation when the leg is used in postural support.

Sudden removal of support often induced active searching movements of the tibia. Reflex responses of motoneurons to chordotonal organ stimulation changed during active searching. All flexor motoneurons showed consistent short latency (18 - 22 msec.) excitatory post-synaptic potentials to chordotonal inputs indicating apparent movement in any direction. These responses were always phasic and resulted in brief, rapid flexion movements. Extensor motoneurons were generally inhibited during flexor excitation. These flexor motoneuron responses may serve an exteroceptive function and produce leg withdrawal if any stimulus impinges upon the tibia when it is raised from a walking surface. Experiments are currently being performed to test these hypotheses and examine the cellular mechanisms underlying changes in reflex mode. Reflex effects of many other proprioceptors may however show similar complex changes during active movements.

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238.11 MOVEMENT DURATION IS CONTROLLED IN SIMILAR WAYS IN LIMB AND SPEECH SYSTEMS. D.J. Ostry*, J.D. Cooke and K.G. Munhall* (SPON: J.D. Brown). McGill University, Montreal, Quebec and University of Western Ontario, London, Ontario.

A central problem in the study of motor control is the identification of variables that are controlled by the nervous system to affect changes in the position of the limbs. As movements differ greatly in their superficial complexity, an important aspect of this problem is whether the nervous system controls motor activities as diverse as limb movements and speech in similar ways. In this paper we have examined this tissue directly by comparing the motor organization of speech with the organization of voluntary movements about the elbow, concentrating on the kinematic regularities that accompany changes in duration. The arm movement study examined kinematic patterns associated with differences in rate and movement amplitude for horizontal movements about the elbow; the speech studies examined kinematic patterns of tongue dorsum and vocal fold movements with changes in speech rate, stress, vowel and consonant. We found that the kinematic patterns for all three articulators were similar. There were reliable correlations between movement amplitude and maximum velocity; the slope of the regression increased with decreases in movement duration. The exact nature of this relationship was studied by plotting on a trial by trial basis the ratio of the maximum velocity to the amplitude of the movement as a function of movement duration. The ratio measure was selected for this purpose because it has been shown to reflect differences in movement trajectory, and in addition, may provide a trial by trial indicator of articulator stiffness. For each of the articulators all changes in the duration of individual movements could be readily accommodated by a single continuous function of the form $V_{max} / A = c / T$, where V_{max} / A is the ratio of maximum velocity to movement amplitude, T is movement duration and c is a constant indicative of a specific velocity profile. The fact that a wide variety of changes in the duration of individual movements can all be accommodated by a single function suggests that the nervous system produces changes in movement duration in different structures in comparable ways. The data also indicate that both for arm and speech systems durational change can be achieved by scalar transformation of the duration and magnitude of velocity profiles.

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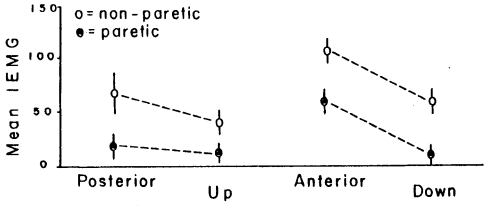
238.12 POSTURAL ADAPTATION FOLLOWING STROKE. R. P. DiFabio, and M. B. Badke*. Postural Control Res. Labs., Univ. of Wisconsin-Madison, WI 53706.

Reaction to unexpected horizontal support surface displacement is facilitated when long latency postural responses stabilize anterior-posterior (AP) sway. Conversely, direct rotation of the ankles requires an attenuation of discharge from lengthened muscle in order to prevent loss of balance. Previous reports have described variations in this phenomenon with age (Forssberg and Nashner, J. Neurosci., 2:545,1982) and with cerebellar degeneration (Nashner, L. M., Exp. Brain Res., 26:59,1976). The purpose of this study was to analyze patterns of postural adapation in three Ss with left cerebrovascular accident ranging from four months to two years duration.

Each S stood on a moveable platform. Perturbations were presented in the horizontal AP plane (5cm/sec) unexpectedly after three rotational (up/down) displacements (8°/sec). Reversals of this sequence were introduced randomly. Averaged surface electromyographs were integrated (IEMG) 100ms from onset and were obtained from tibialis anterior and medial gastrocnemius muscles bilaterally.

Basic facilitation and attenuation of long latency muscle responses were found in all Ss. The figure below shows relative symmetry in adaptation for paretic and non-paretic lower extremities in one S with an infarct localized to the anterior distribution of the left middle cerebral artery. Muscle onset latencies from grouped data provided clear evidence of postural adapations in the non-paretic limb which preceeded those in the paretic extremity ($p < .01$).



Preferential activation of the most stable limb was a consistant feature of balance behavior after stroke. The capacity for both lower limbs to modulate response amplitude was unaltered in this sample.

NEUROMODULATORS I

239.1 **DOPAMINE (DA) AGONIST INDUCED COMPULSIVE BITING (CB) BEHAVIOR IN MONKEYS: ANIMAL MODEL FOR LESCH-NYHAN SYNDROME.** **M. Goldstein and S. Kuga*. New York Univ. Med. Center, Neurochemistry Research Labs., New York, N.Y. 10016.**

We have investigated the effects of DA agonists on tremor and on the occurrence of abnormal involuntary movements (AIM's) in monkeys with unilateral ventromedial (VMT) lesions of the brain stem (Goldstein et al., Science, 179:816, 1973). As an extension of this study, we have now investigated the effects of DA agonists in monkeys with unilateral VMT lesions which were placed 10-12 yrs ago (the monkeys were 2-3 yrs old when the lesion was placed). The administration of L-dopa (100 mg/kg; i.p., in combination with the peripheral dopa decarboxylase inhibitor MK-486 (15 mg/kg; i.p.) or of apomorphine (1-2 mg/kg; i.m.) resulted in a transient relief of tremor with concomitant occurrence of AIMs. The major features of the AIM's were the appearance of choreoathetosis and compulsive licking and biting of the extremities. Pretreatment with fluphenazine (0.2-1.0 mg/kg; i.m.) or with the selective D-1 DA receptor antagonist SCH 23390 (0.2-0.5 mg/kg; i.m.) prevented the occurrence of this behavior. CB behavior was diminshed or completely abolished 5-10 min after administration of SCH 23390 (0.2-0.5 mg/kg; i.m.). These results suggest that CB behavior in monkeys with VMT lesions might be due to stimulation of supersensitive D-1 DA receptors by DA agonists. Thus, monkeys with prolonged supersensitive DA receptors might serve as animal models for the Lesch-Nyhan syndrome, which is characterized by choreoathetosis and CB behavior. Since in Lesch-Nyhan syndrome the deficiency in the HGPRT enzyme might result in a deficiency of brain GTP, it is possible that the modulation of central DA receptors by the nucleotide is abnormal. The defect in the modulation of DA receptors by GTP might also be associated with other disorders of abnormal DA transmission. Studies supported by Grants NIMH 02717 and NINCDS 06801.

239.2 6-METHOXY-TETRAHYDRO-ß-CARBOLINE : A PUTATIVE ENDOGENOUS MODULATOR OF THE 3H-IMIPRAMINE RECOGNITION SITE ASSOCIATED WITH THE SEROTONIN TRANSPORTER. H. Schoemaker*, L.Tahraoui*, T. Tateishi*, A. Segonzac*, C.R. Lee* and S.Z. Langer, Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo, 58 rue de la Glacière, 75013 Paris, France.

Tetrahydro-ß-carbolines (THBC's) inhibit 3H-imipramine binding and serotonin uptake in the brain as well as in platelets (Langer et al., Eur. J. Pharmacol., 1984, 98, 153). 6-Methoxy-THBC was the most potent analog on 3H-imipramine binding. The present experiments further characterize the effect of 6-methoxy-THBC.

3H-Imipramine binding to the rat brain and rabbit and human platelet membranes was measured according to Langer et al. (1984). 3H-5HT and 3H-tryptamine uptake was studied as described before.

The THBC's inhibited with high affinity 3H-imipramine binding in the rat brain with Hill slopes less than unity. 6-Methoxy-THBC was most potent (IC50 = 179 nM) followed by 6-hydroxy-THBC and THBC (IC50 = 825 and 4675 nM). The corresponding open-chain indoleamines had lower affinity. N-Methylation of the open chain indoleamines increases affinity whereas the N-acetylated derivatives are inactive. Dihydro-ß-carbolines have lower affinities than THBC's. In contrast to 3H-imipramine binding in the brain, 3H-imipramine binding to rabbit and human platelets was inhibited with Hill slopes close to unity. Platelet 3H-imipramine binding was most potently inhibited by 6-methoxy-THBC (IC50 = 40 nM) followed by 6-hydroxy-THBC (IC50 = 450 nM). THBC was least potent (IC50 = 1650 nM) Uptake of 3H-5HT and 3H-tryptamine into human platelets was inhibited equipotently with an identical rank order : 6-methoxy > 6-hydroxy > THBC (IC50 = 600, 1300, 4000 nM).

Serotonin and tryptamine affect 3H-imipramine binding through an allosteric mechanism, while for 6-methoxy-THBC the inhibition of 3H-imipramine binding is strictly competitive in human platelet membranes. Thus, in contrast to 5HT and tryptamine, 6-methoxy-THBC,in concentrations up to 100 µM, does not affect the rate of dissociation of 3H-imipramine from its receptor.

These data indicate that 6-methoxy-THBC (endotryptoline), which occurs endogenously in brain as well as in platelets, may be an endogenous ligand acting at the 3H-imipramine recognition site which modulates 5HT uptake. It can not be excluded that, as such, these THBC's may play an important role in affective disorders.

239.3 Ca^{2+} AND cAMP REGULATION OF PROTEIN PHOSPHORYLATION IN THE HERMISSENDA NERVOUS SYSTEM. J.T. Neary, S.A. DeRiemer, L.K Kaczmarek, and D.L. Alkon. Lab. of Biophysics, NINCDS-NIH, MBL, Woods Hole, MA 02543; Depts. Pharm. and Physiol., Yale Univ. Sch. Med., New Haven, CT 06510.

Protein phosphorylation appears to play a role in ion channel activity and in learning and behavior, but little is known about the specific phosphorylation mechanisms that underlie these processes. In Hermissenda, phosphorylation of a 20,000 Mr phosphoprotein (20K PP) is increased following associative learning (Nature 293:658,1981), and ^{32}P incorporation in a 25,000 Mr phosphoprotein (25K PP) is decreased by agents that block the early, transient K^+ current (J. Biol. Chem. 258:8979,1983), a current which is reduced following conditioning (Science 215:693,1982). The purpose of this study is to investigate the phosphorylation enzyme systems in Hermissenda neural tissue and the second messengers that regulate the level of phosphorylation of 20K PP and 25K PP. In vitro protein phosphorylation was studied by incubating [γ-^{32}P]ATP, $MgCl_2$, EGTA and circumesophageal nervous system (CNS) homogenates with $CaCl_2$ or 8-Br-cAMP. Ca^{2+} and cAMP stimulate a minimum of 12 and 19 phosphoprotein bands, respectively, and at least 4 bands are stimulated by both cAMP and Ca^{2+}. CNS proteins are also phosphorylated by exogenous catalytic subunit of cAMP-dependent protein kinase (bovine heart) and by phosphorylase kinase (rabbit skeletal muscle), both purified by E.G. Krebs and associates. Ca^{2+}-stimulated phosphorylation in several bands peaks at about 1 min and declines thereafter; ^{32}P levels in other bands remain relatively constant from 1 to 10 min. Ca^{2+} stimulated bands can be detected at < 1 μM free Ca^{2+}; maximum stimulation for most bands occurs between about 1 and 20 μM free Ca^{2+}. At higher free Ca^{2+} (50-500 μM), phosphorylation of several bands is decreased. The calmodulin blocking drug R24571 inhibits phosphorylation in some, but not all, Ca^{2+}-stimulated bands. The 20K PP and 25K PP appear to be stimulated by cAMP and Ca^{2+}, respectively, but two-dimensional gel electrophoresis and peptide maps are needed to confirm the similarity of the bands labeled in vitro and in vivo. These experiments indicate that Hermissenda CNS contains cAMP- and Ca^{2+}-dependent protein kinases and protein phosphatases which may regulate phosphorylation in 20K PP and 25K PP and that the Ca^{2+}-dependent protein kinase system can respond to increases in intracellular Ca^{++} in the range that occurs in response to extracellular signals. The possible role of these enzymes and their protein substrates in the function of ion channels is a subject for further study.

239.4 ON THE EXISTENCE OF A PUTATIVE ENDOCOID FOR THE ^{3}H-IMIPRAMINE BINDING SITE. M. L. Barbaccia*, O. Gandolfi, D.-M. Chuang* and E. Costa (SPON: M. Hadjiconstantinou). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

In serotonergic terminals, the site that recognizes serotonin (5HT) and the site that binds ^{3}H-imipramine are different but functionally coupled. An endogenous brain modulator (endocoid) in a dose dependent manner selectively displaces specifically bound ^{3}H-imipramine and inhibits the 5HT reuptake (PNAS 80:5134, 1983). This putative endocoid acting on the ^{3}H-imipramine binding site is resistant to pronase and trypsin digestion, has basic characteristics and can be separated from brain 5HT with a cation exchanger resin and with a μ-Bondapak C-18 reverse phase HPLC column. Moreover it is soluble in methanol and ethanol while it is practically insoluble in propanol. The pharmacological and biochemical profile of a number of indolealkylamines or indole-related structures (5-hydroxyindoleacetic acid, tryptophol, 5-hydroxytryptophol, kynuramine, D-L-kynurenine, kynurenic acid, methyl-β-carboline, N-acetyl-serotonin, tryptamine, 5-methoxytryptamine, harmol, harmalol, harmaline, harmine, harmane, norharmane) has been compared with the profile of the endogenous autacoid. None of these indole-related structures inhibited the ^{3}H-imipramine specific binding or the 5HT reuptake in concentrations smaller than 10^{-5}M. In contrast the tetrahydro-β-carboline-related compounds (tryptoline, 6-methoxytryptoline and 6-hydroxy-tryptoline) inhibited ^{3}H-imipramine binding (IC_{50} of 3.0; 0.2; 0.6 μM, respectively) and 5HT uptake. Although the tryptolines appear to be a very interesting class of molecules suitable to be considered as possible candidates for the role of endogenous effector(s) of the ^{3}H-imipramine binding site they show an elution profile from reverse phase HPLC which is different from the one of the putative endocoid which is being extracted from rat brain.

239.5 ENKEPHALIN AND SUBSTANCE P MODULATE DISTINCT SYNAPTIC PROPERTIES OF CHICK CILIARY GANGLION NEURONS. J.F. Margiotta and D.K. Berg. Dept. of Biol., Univ. of Calif., S.D.; La Jolla, CA. 92093.

Preganglionic terminals in the chick ciliary ganglion are known to contain enkephalin- and substance P-like immunoreactive material, though the only known form of chemical transmission through the ganglion is cholinergic. We examined the effects of enkephalin (Enk) and substance P (SP) on ciliary ganglion neurons in cell culture to gain information about their roles in vivo. Neither neuropeptide acted as a conventional neurotransmitter, but each depressed synaptic events associated with cholinergic input to the neurons.

Intracellular recording from ciliary ganglion neurons grown in dissociated cell culture revealed no change in membrane potential or conductance when either Enk or SP at 10-100 μM was applied to cell somata by pressure ejection from a pipet. Enk, however, reversibly depressed the mean amplitude of spontaneous cholinergic postsynaptic potentials (PSPs) that result from synapses formed between the neurons in culture. A reduction of 55 +/- 5% (mean +/- SE, n=12 neurons) was observed with 10-40 μM Enk; the effect was blocked by 10 μM naloxone. Enk had no effect on neuronal acetylcholine (ACh) responses as determined by co-application of ACh and Enk to the cells, suggesting that the effect on transmission might be presynaptic. Consistent with this, Enk (10-40 μM) was found to reduce the duration of the Ca^{++} component of the action potential by 30 +/- 5% (n=14). This effect was also blocked by 10 μM naloxone. A reduction in Ca^{++} current at active nerve terminals would be expected to reduce the amount of ACh released, and thereby result in a depression of PSP amplitude.

In contrast to Enk, SP did have a direct effect on ACh receptor function. Membrane depolarizations induced by prolonged application of 100 μM ACh + 50 μM SP decayed with a half-time of 1.4 +/- 0.2 sec, while responses to ACh alone decayed with a half-time of 2.9 +/- 0.3 sec (n=15). This implies an increased rate of receptor desensitization brought on by SP.

Previous studies in other systems have documented similar effects of Enk on Ca^{++} currents and of SP on ACh receptors. The present results indicate that Enk and SP released from active preganglionic terminals in the ciliary ganglion would be likely to act at pre- and postsynaptic sites, respectively, to diminish cholinergic transmission through the ganglion. (Supported by NS 12601; JFM is an NRS Fellow.)

239.6 EFFECT OF DIALYSIS AND CALCIUM ON TYROSINE HYDROXYLASE FLUCTUATION STABILITY PARAMETERS. Patrick V. Russo*, Arnold J. Mandell. Dept. of Psychiatry, Univ. of California, San Diego, La Jolla, CA 92093.

Tetrahydrobiopterin (BH_4) in addition to its role as co-factor for tyrosine hydroxylase (TH) has been shown to be one of the critical factors in controlling the pattern of kinetic stability of this enzyme (Russo & Mandell, Brain Res., in press 1984). This property of BH_4 has been likened to the effect of the force parameter seen in many nonlinear dynamical systems. This proposed generalized force parameter may also include the net effects of ions, peptides and other molecular agents besides BH_4.

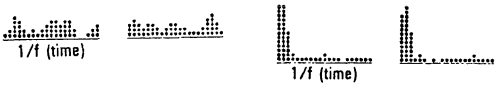
To assess the effect of calcium ion, the 35KG supernatant fraction of rat caudate was first dialyzed for 1 hr in 100 vol of 5 mM potassium phosphate buffer, pH 7.0, and then varying concentrations of calcium were added back, one calcium concentration per experiment. TH velocity was measured radiochemically at 50 points in triplicate, a new point started every 2 min and incubated for 2 min over a 100-min "time course," and the pattern of the time course fluctuations was assessed using metrics from nonlinear dynamics as described elsewhere (Russo & Mandell, Anal. Biochem., in press 1984): e.g. the amplitude (root-mean-square [RMS]), the frequency (power spectral density [PSD]), the variance of the PSD called the spectral entropy [H(f)], and a composite frequency (D) called the fractal dimension were measured.

Dialysis significantly disrupts the organization seen in the fluctuations of normal control preparations at 10 μM BH_4, causing an increase in randomness, with the above measures reflecting this at $p < 0.05$. Adding increasing calcium (up to 25 μM) to the dialyzed preparations systematically brings all the measures of fluctuation stability gradually back to nondialyzed control levels. Calcium is therefore proposed as another of the factors whose combination as a generalized force parameter affects the dynamic stability of TH. This work is supported by DA-00265-11.

239.7 STABILITY PARAMETERS OF RAT RAPHE TRYPTOPHAN HYDROXYLASE: THE EFFECTS OF THYROTROPIN RELEASING HORMONE. S. Knapp and A. J. Mandell. Dept. Psychiatry, Univ. of California, San Diego, La Jolla, CA 92093.

In the presence of saturating concentrations of substrate and cofactor, tryptophan hydroxylase (TPOH; E.C.1.14.16.4) from rat raphe nuclei manifests velocity functions that are characteristically hyperbolic. However, at more nearly physiological, close-to-equimolar ratios of coreactant and enzyme concentrations, measures across small (0.5 μM) intervals of tetrahydrobiopterin (BH_4), substrate (TRP), or time become unstable, developing reiterative asymptotic zones (Knapp & Mandell, J. Neural Transm. 45:1, 1979) similar to the intermediary plateaus noted by others in substrate saturation curves of other regulatory enzymes. The kinetic instabilities of rat raphe TPOH are consistent with the presence of multiple metastable states, as demonstrated by both thermal and storage inactivation techniques applied to the brain enzyme and transitions among three activity states that were sensitive to changes in reducing conditions, observed in mouse mastocytoma TPOH.

Thyrotropin releasing hormone (TRH) and its receptors are located in both central serotonergic and dopaminergic systems making it a candidate for a role in regulating the stability of a representative brain mixed-function oxygenase such as TPOH. Among the statistical analytical approaches to the far-from-equilibrium kinetic data are the power frequency spectral transforms from two experiments each performed in the absence (left) and presence (right) of 3 μM TRH across time. The more random appearance of the control populations is shown by the multiple broadband power peaks in the spectrum, consistent with the simultaneous expression of multiple dynamical modes. The TRH influence is seen as a a single broad band on the slow side of the spectrum, representing a stabilized population having reduced degrees of freedom. This research is supported by DA-00265-11.



Control 3 μM TRH

239.8 AN IN VIVO SEMIDERIVATIVE ELECTROANALYSES OF DYNORPHIN(1-13) ON DOPAMINE AND SEROTONIN RELEASE FROM RAT STRIATUM. P.A. Broderick, Departments of Psychiatry and Neuroscience, The Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, New York 10461.

Dynorphin (1-13) is a potent opioid peptide (Goldstein et al., Proc. Natl. Acad. Sci., 76:666, 1979). Dynorphin(1-13) has been shown to substitute for morphine in morphine-dependent monkeys. Yet, the opioid peptide was incapable of producing morphine-like overt effects in non-tolerant monkeys, thus perhaps potentiating morphine effects only in tolerant animals (Aceto et al., Eur. J. Pharmacol., 83:139, 1982). The purpose of the present paper was to study the underlying neurochemistry of the peptide, dynorphin(1-13).

The effect of dynorphin(1-13) on the simultaneous release of the neurotransmitters dopamine and serotonin from striata of male, Sprague-Dawley rats was studied by semiderivative electroanalyses, an in vivo electrochemical methodology. Chloral hydrate, anesthetized rats (body temperature maintained at 37° C) underwent stereotaxic surgery for positioning of a teflon-coated working electrode (150-175μ)(stearate modification, Blaha and Lane, Brain Res. Bull., 10:861,1983) in anterior striatum. A Ag/AgCl reference electrode and Pt auxiliary electrode was placed in contact with the rat cortex. Semiderivative voltammograms were recorded every ten min. Potentials were applied between -200 and +500 mv, at a scan rate of 10 mv sec^{-1}. Calibration of the working electrode took place in physiological phosphate buffer solution.

The results showed that dynorphin(1-13)(1.5 mg/kg sc) decreased the dopamine signal 39% below that signal produced by endogenously released dopamine. Simultaneously, dynorphin(1-13) increased the serotonin signal 40% over baseline values. The opposing effects of dynorphin(1-13) on dopamine and serotonin release occurred gradually over a three hour time period. Preliminary data from studies using a lower dose of dynorphin(1-13)(0.4 mg/kg sc) showed a smaller (18%) decrease in dopamine release and little or no effect on serotonin release from rat striatum. Saline had no effect on endogenously released striatal dopamine and serotonin.

These data are consistent with previous suggestions that dynorphin(1-13) may function as a modulatory peptide. These data further suggest that this modulatory role may take place through neurotransmitter release regulation.

Supported by USPHS Grant MH15788.The author is grateful to Dr. N. Lee, U. of Calif., San Fran., for dynorphin(1-13).

239.9 PURIFICATION AND CHARACTERIZATION OF AN ENDOGENOUS MODULATOR WITH ACTIVITY AT BENZODIAZEPINE RECEPTORS. L. Antonian*, B. Beer, A.S. Lippa*. Dept. of CNS Res., Medical Research Division of American Cyanamid Co., Lederle Labs, Pearl River, NY 10965.

Specific receptors for benzodiazepines exist in the brain and in peripheral tissue. The identity of the physiological ligand(s) for the benzodiazepine receptors remains equivocal, although numerous endogenous substances which act on the benzodiazepine receptor have been isolated from various tissues and biological extracts.

We have isolated a highly potent substance from human and rat serum which competitively and in a dose dependent manner inhibits binding of [^{3}H]-flunitrazepam. A 50% inhibition of 0.25 nM [^{3}H]-flunitrazepam is achieved with 25 μl of serum. Based on Scatchard analyses 50 μl of serum is equipotent with 2 nM clonazepam in reducing the affinity of [^{3}H]-flunitrazepam 1.5-2 fold . Generally, 80 units of bioactivity (10% inhibition of [^{3}H]-FLU binding equals one unit of bioactivity) per mL of serum is observed. The potency of this substance is not affected by GABA modulation of [^{3}H]-FLU binding. This factor has been purified 12-fold from serum using a Blue Sepharose CL-6B affinity column. The isolated complex, "albumin and active substance", retains full activity at [^{3}H]-FLU binding sites (~80 units of activity per mL of purified complex). This affinity purified complex is also potent at peripheral BDZ receptors as measured by inhibition of [^{3}H]RO5-4864 binding. The bioactivity of "albumin and active substance" is resistant to trypsin digestion.

Chemical modification of the affinity purified complex with dithiothreitol separates the active substance from albumin which is then chromatographically purified by HPLC on molecular sieving and reverse phase columns.

This purified endogenous modulator, although as yet of an unknown molecular nature, is active at BDZ receptors but may play a more general role in membrane receptor function.

240.1 EFFECT OF LESIONS OF THE NUCLEUS OF THE TRACTUS DIAGONALIS (TD) ON PROTEINS IN THE HIPPOCAMPUS AND OCCIPITAL CORTEX AS ANALYZED BY TWO-DIMENSIONAL GEL ELECTROPHORESIS (2DE).
W.E. Heydorn, K.Q. Nguyen*, G.J. Creed* and D.M. Jacobowitz. Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

2DE is a method by which complex mixtures of proteins can be resolved based upon differences in mass and charge. When combined with highly sensitive staining techniques and computer-assisted densitometry, the relative concentration of each protein present on the gels can be determined. Recently, we have embarked on a series of studies designed to ascertain which proteins are regulated by the major neurotransmitter systems in the CNS. Since the td provides the major cholinergic input to both the hippocampus (H) and the occipital cortex (OC) (Brain Res. Bull. 10:365, 1983), we decided to electrolytically ablate the td and examine protein concentrations in these two brain areas using 2DE. Rats received either a sham or an electrolytic lesion of the td. Nine or 35 days later, the animals were killed and tissue was obtained from the H, OC and caudate nucleus (CN) for determination of both choline acetyltransferase (ChAT) activity and for separation of proteins by 2DE. The td lesion produced a significant reduction in ChAT activity in both the H (55%) and the OC (43%), while having no effect on enzyme activity in the CN, a region not receiving its cholinergic input from the td. Of the 140 proteins analyzed quantitatively, only four were found to be altered in concentration in both the H and the OC after the td lesion. Protein 82 (MW 39 Kd, pI 6.5) was reduced 71% in the H and 50% in the OC nine days after the lesion, while protein 109 (MW 32 Kd, pI 6.4) was elevated 140% in the H and 130% in the OC at the same time point. Thirty-five days after the lesion, the concentration of both these proteins had returned to control levels. A third protein, designated as No. 6 (MW 58 Kd, pI 5.7), was unchanged in concentration nine days after the lesion, but was elevated 44% in the H and 27% in the OC 35 days after lesion. The fourth protein affected by the lesion, No. 74 (MW 39 Kd, pI 5.8), was significantly elevated in concentration both 9 and 35 days after the lesion in the OC, but only at day 35 in the H. In all cases, no effect of the lesion was detected in the CN. These results suggest that the concentration of these 4 proteins in brain may be regulated by the cholinergic input to these brain areas. The possibility exists that one or more of these proteins in brain may be related to the muscarinic cholinergic receptor.

240.2 EFFECTS OF DIETARY CHOLINE SUPPLEMENTATION ON THE BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF PENTOBARBITAL.
L. Wecker and G. Cawley*. Department of Pharmacology, Louisiana State Univ. Med. Ctr., New Orleans, LA 70112.

Chronic supplementation with choline to rats increases the levels of choline in blood, does not alter the concentration of choline or acetylcholine (ACh) in brain, but markedly alters the responsiveness of cholinergic neurons to pharmacological manipulation. We have postulated that these effects are not mediated by specific alterations in the cholinergic system, but rather reflect a non-specific effect of choline on neuronal membranes. To further characterize the effects of choline supplementation, we investigated the effects of dietary choline availability on the behavioral and neurochemical effects of pentobarbital (PB, 50 mg/kg, i.p.). Male Sprague-Dawley rats (initial weight 180-200g) were maintained on either basal choline chow (0.2 % choline chloride) or choline-supplemented chow (2.0 % choline chloride) for 28-32 days. The behavioral effects of PB were assessed by measuring the time of loss of righting reflex and the neurochemical effects assessed by quantitating changes in the concentration of ACh in brain. The sedative/hypnotic effects of PB were evident in 80% of rats maintained on the basal chow and the duration of this effect was 60-100 min. Rats fed the supplemented chow were somewhat resistant to the effects of PB. Only 25% of the rats lost their righting reflex, and the duration of this effect was 60-75 min. In rats that had lost their righting reflex, the levels of ACh (30 min after injection of PB) increased by 25%, 65%, and 100% in striatum, hippocampus, and cortex, respectively, regardless of which dietary regimen the rats were fed. Similarly, in alert rats that had been injected with PB, no change in the levels of ACh were noted in the hippocampus or cortex, despite the different diets. Results indicate that supplementation with choline does not alter the cholinergic response of brain neurons to PB, but does significantly attenuate the behavioral response.
(Supported by USDHHS grant NIMH 33443.)

240.3 FACTORS AFFECTING THE SIZE OF CHOLINERGIC NEURONS IN THE BASAL FOREBRAIN OF THE RAT: EVIDENCE FOR PLASTICITY.
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In previous studies we examined the response of cholinergic neurons in the basal nucleus of Meynert (BN) to damage of the cerebral cortex upon which they project. Following unilateral damage, ipsilateral cells shrink but do not die[1] and contralateral cells hypertrophy (in press). Following bilateral damage, cells on both sides shrink but do not die. In order to study the factors underlying these changes, further experiments have been conducted. Fifty-two Wistar rats were used. Experimental rats and their matched controls were perfused, the brains processed for immunohistochemical detection of choline acetyltransferase (ChAT) and the cross sectional areas of BN cells measured on both sides[1]. The importance of age at operation and survival time on the hypertrophy of cells on the side opposite to unilateral cortical damage was examined in littermate pairs. It was greatest when animals were operated on when immature, but occurred at all ages. It was present by seven days, was maximum by one month, and persisted after a year postoperatively. The importance of commissural projections to these changes was examined by transection of the corpus callosum. This resulted in a hypertrophy of cells on both sides of the BN of a similar scope and dimension to that previously seen. The effect of destruction of cortical neurons without damage to cholinergic afferents was examined by application of kainic acid unilaterally to the cortex. This resulted in shrinkage, but not death of ipsilateral cells, in a manner identical to cortical destruction. However, in contrast to cortical destruction, there did not appear to be any hypertrophy of contralateral cells. These findings indicate that cholinergic cells of the BN will undergo pronounced changes in cell size under a variety of circumstances. Of particular interest is their capacity to hypertrophy. In other systems, neuronal hypertrophy has been correlated with terminal sprouting[2]. In this system, recovery with time of cortical ChAT activity has been reported following subtotal lesion of the BN[3]. Together these findings suggest a marked capacity for plasticity of these neurons.

1 Sofroniew, M.V. et al., Brain Res. 289 (1983) 370-374.
2 Goldschmidt & Steward, J.Comp.Neurol. 189 (1980) 359-379.
3 Wenk, G.L. & Olton, D.S., Brain Res. 293 (1984) 184-186.

240.4 INTRACELLULAR ANALYSIS OF FUNCTIONAL CONNECTIONS BETWEEN TRANSPLANTED SEPTAL CHOLINERGIC NEURONS AND HOST HIPPOCAMPUS
M. Segal,* Center for Neurosciences, The Weizmann Institute of Science, 76100 Rehovot, Israel (SPON: E. Hazum).

Acetylcholine (Ach) triggers a number of postsynaptic responses in hippocampal neurons. These include activation of a transient K^+ current, blockade of a non-inactivating K current activated by depolarization (Im), and blockade of Ca dependent K current (Ic) and perhaps other K currents as well. Calcium, temperature and membrane potential determine some effects of Ach. The slice preparation, where these observations were made, lacks an intact cholinergic pathway. Thus, a comparison between effects of Ach and activation of a cholinergic synapse cannot be made directly. The transplantation of septal neurons into a host hippocampus can provide such a test system.

The septal nuclei of embryonic rats were dissociated mechanically under sterile conditions. Cell suspensions were injected stereotaxically into the hippocampus of fornix transected adult rats, according to the method of Bjorklund et al. (Acta Physiol. Scand. Suppl. 522: 1-7, 1983). The host rats were sacrificed some 1-3 months after the operation and the hippocampus sliced into 350μm transverse slices. Intracellular recording was made from CA1 neurons adjacent to the graft. Electrical stimulation of the graft produced a voltage dependent depolarization in some recorded neurons. This was associated with an increase in spontaneous and anodal break action potential discharges. In addition, a hyperpolarizing response which typically follows a burst discharge was blocked during the depolarization indicating that the stimulation may block Ic. The effects of the stimulation were antagonized by atropine. A response to the stimulation was seen two weeks but not one week after grafting. Over time, cells that were located away from the graft became activated by the stimulation. This was correlated with the extent of proliferation of acetylcholine-esterase containing fibers around the graft. Some of the effects of graft stimulation were mimicked by topical application of Ach.

These results suggest that grafted septal neurons make viable cholinergic connections with a host hippocampus.

240.5 PHYSIOLOGY OF CORTICALLY PROJECTING NEURONS IN MONKEY NUCLEUS BASALIS OF MEYNERT. G. Aston-Jones[1], J. Rogers, S. Grant[2], M. Ennis*[1], R. Shaver*[1] and R. Bartus[3]. Dept. Neurology, U. Mass. Med. Cntr., Worcester, MA 01605; [1]Dept. Biology, NYU, New York, NY 10003; [2]Dept. Psychiatry, Yale Univ. Med. Sch., New Haven, CT 06508; [3]Lederle Labs., Pearl River, NY 10965.

A direct cholinergic projection to cerebrocortex from basal forebrain (nucleus basalis of Meynert in primates) (nBM) has been demonstrated for many species. This system is of interest both because it is a nonthalamic source of widespread cortical innervation (similar to catecholamine and indolamine systems) and because nBM degeneration is implicated in Alzheimer's Disease. We recently reported on the physiology of putatively cholinergic nBM neurons in young adult and aged rodents (Aston-Jones et al., Neurosci Abst, 1983; Neurosci Lett, 1984; Brain Res, in press; Rogers et al., Neurosci Abst, 1983). In the present study, we investigated this system in young adult monkeys.

To date, single unit nBM recordings have been obtained in 3 C. appella monkeys under halothane anesthesia. Cortically projecting neurons were identified by antidromic activation (verified with high-frequency driving or collision testing). Fifteen histologically localized nBM neurons met antidromicity criteria, with 13 driven from frontal and 2 from somatosensory cortex. Spontaneous discharge patterns varied among cells; in fact, many driven cells were nonspontaneous.

The range of conduction latencies was 4.6-39.0 ms (median = 8.0 ms) in prefrontal cortex, and 6.2-31.0 ms (median = 17.0 ms) in midfrontal cortex. Based on estimated fiber lengths of 28 and 43 mm, respectively, these latencies translate to conduction velocities of 0.7-6.9 m/s. Such values suggest that many nBM fibers are myelinated. Conduction latencies for somatosensory cortex ranged from 33.0-84.0 ms (median = 61.5 ms). Most cells exhibited multiple antidromic latencies from single stimulation sites. Indeed, for many cells the driven spike "jumped" between two constant latencies on successive stimuli. In addition, 12 of 14 cells tested were antidromically activated from different cortical sites separated by 2-12 mm mediolaterally. These results, similar to those obtained in rodents, indicate that nBM fibers branch substantially within frontal and somatosensory cortex.

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240.6 CENTRAL CHOLINESTERASE INHIBITION AND BEHAVIORAL DEPRESSION PRODUCED BY INTRASTRIATAL DFP. M.R.Lynch, M.A.Rice* and S.E. Robinson. Dept. of Pharmacology and Toxicology, Medical College of Va., Richmond, Va. 23298 (SPON: M.J.Kallman)

Central AChE inhibition was induced by bilateral infusion of DFP (40.75, 81.5 or 163nmol) dissolved in 1:9 emulphor: CSF vehicle and measured by the method of Ellman et al.(Biochem. Pharm.,7:88,1961). Each dose was evaluated at 20 min, 1 hr and 24 hr after intrastriatal administration in unanesthetized rats. In addition, spontaneous locomotor behavior was measured at all three time points for the 81.5 and 163 nmol doses. Stereotaxically implanted guide cannulas were aimed at AP+7.2,L±3,DV-3 (Konig & Klippel) 72 hr prior to drug or vehicle infusion. Tubing connected internal cannulas to two 50µl Hamilton syringes, simultaneously driven by an infusion pump at 1 µl/min. Spectrophotometric determinations of acetylthiocholine hydrolyzed/min were made with homogenates from bilaterally pooled striata, parietal cortices, hippocampi, amygdaloid complexes and the hypothalamus and medulla/pons, as well as from fresh trunk blood.

Significant enzyme inhibition was produced by all doses at all times for the striatum, medulla/pons and parietal cortex ($\bar{X}$=40% of control for the striatum vs 59-70% for other areas with 81.5nmol at both 20 min and 1 hr). All but the 20 min measure for 40.75nmol yielded significant inhibition in the hypothalamus and hippocampus (68-70%, as above), with the amygdala showing a different pattern for all measures. Trunk blood was significantly lower only at 1 hr with 163nmol and 24 hr with 81.5nmol. Examination of the % vehicle-control values for each brain area revealed significantly lower %s for the high dose in all areas. Although this dose showed a trend toward enzyme recovery over time, this was not significant.

Locomotor behavior, measured in 5 min intervals over 30-min sessions, was determined by the number of activity counts from capacity-coupled platforms. Significant depressions were noted with the 163nmol dose at 20 min and 1 hr, when there was an overall inhibition of AChE in all brain areas ($<$40% for all but amygdala at 1 hr). Activity was normal at 24 hr however, while enzyme inhibition remained significantly reduced. For 81.5nmol, there was significant behavioral depression only at 20 min. This is interesting in light of the fact that enzyme activity in the striatum was twice that observed after the 163nmol dose (38 vs 20%), while all other areas remained at $>$70% control values (except for the parietal cortex at 60%). (Supported by DAMD17-83-C-3183 from the USAMRDC).

240.7 ACETYLCHOLINESTERASE FROM THE SKELETAL MUSCLE OF THE LAMPREY, PETROMYZON MARINUS. Leo Pezzementi. Depts. of Biology and Chemistry, Oberlin College, Oberlin, Ohio 44074

The lamprey is considered to be one of the most primitive surviving vertebrates. Acetylcholinesterase hydrolyzes acetylcholine at the neuromuscular junction of vertebrates, and thus plays an important role in neuromuscular transmission. A biochemical study of the acetylcholinesterase from the skeletal muscle of the lamprey, Petromyzon marinus, could provide evidence concerning the evolution of acetylcholinesterase in vertebrates.

I found that extracts of lamprey skeletal muscle hydrolyzed acetylthiocholine at a rate of 0.83 nmoles min^{-1} mg^{-1}(SD = 0.23 nmoles min^{-1} mg^{-1}, N = 11). Optimal extraction of acetylcholinesterase activity was obtained in 10 mM $NaHPO_4$, pH 7.2, 0.25 mM EDTA, 150 mM NaCl, and 0.5% Triton X-100. The apparent Km of the acetylcholinesterase activity was 31 µM (SD = 10.1 µM, N = 3), and the enzyme was inhibited by excess substrate. The acetylcholinesterase activity was also inhibited by BW 284c51, an inhibitor of acetylcholinesterase (IC_{50} = 5.6 nM, SD = 1.5 nM, N = 5). However, iso-OMPA and ethopropazine, inhibitors of pseudocholinesterase, did not inhibit the acetylcholinesterase activity until high concentrations of drug were reached. These data indicate that the acetylcholinesterase activity of lamprey skeletal muscle is due to acetylcholinesterase and not to pseudocholinesterase.

Velocity sedimentation on sucrose gradients revealed a single molecular form of acetylcholinesterase with an apparent sedimentation coefficient of 8.3 S (SD = 0.01 S, N = 3). This is unusual for vertebrate skeletal muscle. I performed these sedimentation velocity experiments with frozen (-80° C) muscle extracted in buffers that lacked protease inhibitors. I plan to repeat and extend these experiments with fresh muscle extracted in the presence of protease inhibitors.

No ^{125}I-α-bungarotoxin binding to extracts of lamprey skeletal muscle was detected. This research was supported by a grant from Oberlin College.

240.8 PRE- AND POSTSYNAPTIC ACTIONS OF ACETYLCHOLINE (ACh) IN THE CINGULATE CORTEX IN VITRO. D.A. McCormick and D.A. Prince. Dept. Neurol., Stanford Med. Ctr., Stanford, CA 94305.

Application of ACh to neurons of the motor-sensory cortex or hippocampus causes a long latency muscarinic slow depolarization and increase in input resistance (R_N). ACh also has presynaptic actions in hippocampus where it reduces the amplitude of EPSPs and IPSPs on CA1 pyramidal neurons, however such effects have not been examined in the neocortex. We therefore studied the actions of ACh on neurons of guinea pig cingulate cortex slices, maintained in vitro at 36°C.

Recordings from 185 layer V cells were obtained during application of ACh (5-20 mM) microdrops from a 5-10µ pipette near the recording electrode. At resting membrane potential (V_m) ACh evoked a short (< 0.5 sec) latency barrage of depolarizing PSPs; a decrease in R_N; a decrease in the amplitude of orthodromic PSPs; but no slow depolarization. In contrast, when neurons were depolarized from rest by 10-15 mV with DC current, ACh elicited a short latency hyperpolarization, a barrage of depolarizing and hyperpolarizing PSPs, and a decrease in R_N in nearly all cells. This was followed after 3-5 sec by a long lasting depolarization and repetitive spiking in about 50% of cells. All responses were blocked by atropine (10^{-6}M) bath perfusion. The early hyperpolarizing response had an average reversal potential of -75.8 mV, which was similar to that for orthodromic IPSPs (-69.6 mV) and hyperpolarizing responses produced by GABA application. The PSPs and the hyperpolarizing ACh response were blocked by bath application of 3 mM Mn^{++}, 0.5 mM Ca^{++}, or TTX (1 µM), while the late depolarization was not. The early hyperpolarization and spontaneous hyperpolarizing PSPs seen in depolarized neurons were blocked by bicuculline or picrotoxin (50 µM), although EPSPs could still be evoked by ACh application. In TTX, ACh could elicit slow depolarizations and superimposed spikes probably mediated by Ca^{++}. IV plots in TTX showed that ACh produced a voltage-dependent increase in R_N at V_ms < -65 mV. Neurons with slow depolarizing responses were identified as pyramidal type cells with Lucifer Yellow. Our data are consistent with the hypothesis that ACh has two effects: 1) a rapid depolarization of GABAergic (and other) neurons or their terminals which causes an initial barrage of PSPs, concurrent with presynaptic inhibition due to occlusion or terminal depolarization; and 2) a slow decrease in voltage-dependent K^+ current (M-current?) in pyramidal cells. Supported by NIH grants NS 12151 and NS 06477 from the NINCDS (DAP) and a fellowship from the CCHD (DAM).

240.9 ACETYLCHOLINE SYNTHESIS AND RELEASE IN THE PRESENCE OF A COMPOUND THAT BLOCKS UPTAKE OF TRANSMITTER BY SYNAPTIC VESICLES. B. Collier* and S.A. Welner* (SPON: B.A. Esplin). Dept. Pharmacol., McGill University, Montreal H3G 1Y6, Canada.

The compound 2-(4-phenylpiperidino) cyclohexanol (AH5183) blocks the ability of cholinergic synaptic vesicles to accumulate acetylcholine (ACh). To measure the transmitter store pre-packaged in releasable vesicles, the release of ACh was measured from the perfused superior cervical ganglion of the cat during 5Hz stimulation of the preganglionic nerve. In the absence of AH5183, release was well maintained, but in the drug's presence it was not. During perfusion with AH5183, evoked ACh release in the first 2 min of stimulation was normal, but subsequently it declined to zero within 10-12 min. The total amount of ACh released in the presence of AH5183 was 194 ± 9 pmoles; the initial store of ACh in these ganglia was 1378 ± 61 pmoles. Thus, some 15% of the transmitter store is available for release in the presence of AH5138, as if 85% of tissue ACh is in a store other than readily releasable synaptic vesicles.

To test the effect of AH5183 on ACh synthesis, we measured transmitter release and final tissue content. ACh synthesis was activated during activity by a similar amount in the presence (78 ± 3 pmoles/min) or in the absence (83 ± 10 pmoles/min) of the drug; as AH5183 depressed release, the synthesized ACh increased tissue content. Thus, it is apparent that, if AH5183 blocks vesicle uptake of ACh, that process does not regulate ACh synthesis. When transmitter release was depressed by Mg^{++}, ACh synthesis was not increased during stimulation and tissue content remained normal. Measures of uptake and acetylation of diethylhomocholine complemented studies of ACh turnover. This choline analogue is taken up by the nerve terminal choline uptake mechanism, some of it is acetylated, but the ester is not taken up by synaptic vesicles and is not releasable. Preganglionic stimulation increased the uptake (4-fold) and the acetylation (7.5-fold) of diethylhomocholine; AH5183 had no effect on these measures; but Mg^{++} blocked the enhanced acetylation without effect on the enhanced uptake of the choline analogue. Mg^{++} blocks Ca^{++} influx whereas AH5183 does not; thus, it is concluded that increased precursor delivery can increase ester production if activity also increases Ca^{++} influx.
(Supported by MRC of Canada).

240.10 Monoclonal Antibody Assisted Purification of Rat Brain Choline Acetyltransferase. G.D. Crawford*[1], L. Correay*[2] and P.M. Salvaterra[2]. [1]Dept. of Neurol., Baylor Coll. of Med., Houston, Tx. and Beckman Research Inst. of City of Hope, Neurosci. Div., Duarte, CA.

Choline acetyltransferase (ChAT;E.C. 2.3.1.6) may be purified in high yields from rat brain extracts by a simple two stage procedure. A well-characterized non-inhibitory monoclonal antibody 1E6 (Crawford et al, PNAS 79:7031-7035, 1982)) was coupled to Sepharose 4B and used to quantitatively adsorb ChAT enzyme activity from ammonium sulfate concentrated fractions. The enzyme activity remained adsorbed to the resin after exhaustive washing with high salt buffers. In several preparations 70-80% of the enzyme activity present in the ammonium sulfate fraction, and 40-60% of the total activity overall was recovered as Sepharose-antibody immobilized enzyme. The adsorbed enzyme was efficiently eluted under denaturing conditions and an analysis by SDS polyacrylamide electrophoresis indicates that the product contains primarily (≈90%) a 68,000 dalton polypeptide. The remaining species are divided among 54,000, 40-36,000, 18,000 and 13,000 dalton fragments with some unexplained material at the electrophoretic front even in high density gels. This pattern is reminiscent of that determined for Drosophila melanogaster ChAT purified by classical biochemical stratagies. As in this case, inclusion of the protease inhibitors pepstatin and trisalyl during the extraction did not reduce the complexity of the electrophoretic pattern. The relationship of these peptides to the 68,000 dalton protein is currently being examined by peptide mapping.

A similar, if not identical, material was purified from brain extracts using a second monoclonal antibody, 4G5 obtained from the same panel as 1EG. Protein in the range of 35-40 µg can be obtained from 175 gm tissue at an overall efficiency of 45%, which allows an estimate of purification in excess of 200,000 fold. Membrane bound ChAT was also purified by an identical procedure. The source was a detergent extract of brain residue previously washed to constant specific activity. This material yields a product similar to the soluble form, but differing quantitatively in the relative amounts of 68,000 and low molecular weight polypeptides. Research supported by NA19481 and the Harkins Foundation 470G011435.

240.11 DIFFERENT POPULATIONS OF RAT CORTICAL NEURONS STAIN FOR CHOLINE ACETYLTRANSFERASE AND ACETYLCHOLINESTERASE. B.H. Wainer [a], A.I. Levey[a], D.B. Rye [a], A. Bluemke [a], E.J. Mufson[b], M.-M. Mesulam [b]. The Departments of Pathology and Pediatrics, The Univ. of Chicago, Chicago, IL 60637[a]. Dept. of Neurology, Harvard Univ., Beth Israel Hospital, Boston, MA 02215[b].

Although controversy has existed over the presence of intrinsic cortical cholinergic innervation (see Wainer et al. Neurochem. Intl.6, 163, 1984), choline acetyltransferase (ChAT) immunoreactivity has been localized to small cortical neurons in the rat (Houser et al Brain Res., 266, 97, 1983). We further studied these neurons using ChAT immunohistochemistry, acetylcholinesterase (AChE) histochemistry and co-localization of ChAT and AChE. ChAT localized mainly to small upper-layer bipolar neurons in isocortex, and small multipolar neurons stained in allocortex. AChE was seen in polymorphic cells throughout all layers. Combined staining for ChAT and AChE localized the markers to different cell populations. Colocalization of GABA-immunoreactivity and AChE, however revealed some double-labeled cells. Possible connections of the cortical ChAT-immunoreactive cells were studied in rats receiving injections of horseradish peroxidase-wheatgerm agglutinin (HRP-WGA) into either cortex or brainstem. Using a technique for co-localizing ChAT and tracer (Wainer and Rye, J. Histochem. Cytochem. 36, 439, 1984), ChAT and retrograde HRP-WGA localized to different neuronal populations. We conclude that cortical cholinergic cells are not visualized by AChE and are most likely local circuit neurons. Some AChE-positive cortical neurons are likely to be GABAergic. Supported by USPHS HD-04583, NS-17661, the McKnight Foundation (B.H.W.); 5-T32GM07281 (D.B.R. and A.B.); NS-09211, NS-07011 and Essel Foundation (M.M.-M.).

241.1 PRO-SOMATOSTATIN-RELATED PEPTIDES ALTER THE DISCHARGE RATE OF RAT CORTICAL AND HIPPOCAMPAL NEURONS IN VIVO; AN IONTOPHORETIC STUDY. G.R. Siggins and E.D. French. Div. Preclin. Neurosci. and Endocrin., Research Institute of Scripps Clinic, La Jolla, CA 92037, and Maryland Psychiatric Research Center, Baltimore, MD 21228.

Previous immunohistochemical studies suggest the presence of two pro-somatostatin related peptides, somatostatin 28 (SS28) and SS28(1-12), as well as the original SS14 or SS28(15-28), in fibers and cell bodies of cerebral cortex (Morrison et al, Brain Res. 262:344, 1983) and hippocampus (Morrison et al, Neurosci. Lett. 34:137, 1982). Furthermore, SS28(1-12) can be released from brain slices by a Ca++-dependent mechanism (Bakhit, et al Nature, 301:524, 1983), suggesting a possible neurotransmitter role. Therefore, the present study was designed to test the effects of SS28 and SS28(1-12) on single spontaneous and acetylcholine-activated neurons in parietal cortex and dorsal hippocampus.

Standard extracellular recording and iontophoretic methods were used in halothane anesthetized rats (n=18). SS28 and SS28(1-12) were dissolved in normal saline (3 mM; pH 6.5) and applied from 5-barrel micropipettes by electroosmosis.

Of the 54 tests on 42 cortical neurons evaluated, we found that both pro-somatostatin peptides were predominately inhibitory: SS28 inhibited 67% and excited 12%; SS28(1-12) inhibited 40% and excited 12%. In 49 tests on 33 hippocampal neurons, SS28 was more often excitatory, whereas SS28(1-12) was predominantly inhibitory: SS28 inhibited 6% and excited 81% of hippocampal neurons; SS28(1-12) inhibited 52% and excited 26% of these neurons. Interestingly, a tachyphylaxis to the initial inhibitory actions of SS28 was observed in approximately 70% of cortical cells. This phenomenon did not occur with SS28(1-12). Also, when SS28 and SS28(1-12) were compared on the same neuron (7 in cortex, 10 in hippocampus), both produced responses in the same direction; generally, SS28 had a longer and more pronounced action than the smaller SS28(1-12).

These results suggest that both SS28 and SS28(1-12) have electrophysiological effects on central neurons; they might thus serve as peptide messengers in these brain areas, perhaps independently of SS14 or as co-transmitters. Current studies are aimed at comparing the responses and possible interactions of these two peptides with SS14. Supported by the USPHS (AM 26741).

241.2 HYPERRESPONSIVENESS OF THE MESOLIMBIC DOPAMINE SYSTEM AFTER REPEATED PEPTIDE INJECTION INTO THE VENTRAL TEGMENTAL AREA. P. W. Kalivas* (SPON: B. V. Updyke) Dept. Pharmacology, LSU Med. Ctr., New Orleans, LA 70112

Acute microinjection with neurotensin (NT), substance P (SP) or the enkephalin analogue, D-Ala$_2$-Met$_5$-enkephalinamide (DALA), into the ventral tegmental area (VTA) of the rat activates the mesolimbic dopamine (DA) system. This is shown by peptide-induced increases in DA metabolites in the nucleus accumbens and locomotor activity. In this study, male S.D. rats were stereotaxically implanted with chronic bilateral injection cannulae over the VTA. One wk after surgery, rats received one intra-VTA microinjection per day for 5 days with either NT (1.0 µg), DALA (1.0 µg), substance P (10.0 µg) or saline. Immediately after microinjection the rats were placed in a photocell apparatus and locomotor activity measured for 120 min. While all three peptides elicited a locomotor response greater than saline, the locomotor response was progressively enhanced after each progressive injection with DALA and NT, but not SP. Further, rats which had received daily injection with DALA or NT demonstrated an enhanced response to acute peptide injection up to 2 wks after daily treatment. This progressive enhancement of locomotor activity was associated with an enhanced DALA or NT-induced increased in DA metabolites in the nucleus accumbens. These data indicate that daily injection with some endogenous peptides into the VTA can result in a mesolimbic DA system that is hyperresponsive to pharmacological stimulation. This conclusion is supported by the observation that rats receiving daily intra-VTA injection with DALA or NT demonstrated an enhanced motor response to amphetamine, a drug which produces increased locomotor activity via increasing mesolimbic DA release, but not caffiene, which acts via a non-DA mechanism to increase motor activity.

241.3 EFFECT OF SYNTHETIC HUMAN PANCREATIC GROWTH HORMONE RELEASING FACTOR ON SOMATOSTATIN RELEASE IN VITRO. M.C. Aguila* and S.M. McCann. Department of Physiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

The effect of synthetic human pancreatic growth hormone releasing factor (hpGRF-40) on somatostatin (SRIF) release from the median eminence (ME) of the hypothalamus, was evaluated using an in vitro incubation system. Adult male rats were used as tissue donors. The MEs were first preincubated in 0.4 ml of Krebs-Ringer bicarbonate-glucose buffer, pH 7.4, at 37°C, in an atmosphere of 95% O_2, 5% CO_2, with constant shaking. After 30 min preincubation, medium was discarded and replaced by medium containing various concentrations of hp GRF-40, or hpGRF-40 plus either Pimozide or dopamine (DA). HpGRF-40 stimulated SRIF release in a dose-related manner. This effect was significant at concentrations varying from 10^{-11} to 10^{-7}M. Maximal stimulation was observed at 10^{-10}M. To determine if the effect of hpGRF-40 on SRIF release was mediated by DA, Pimozide was added in vitro at a concentration of 10^{-6}M, and the effect of hpGRF-40 was then evaluated. Pimozide did not alter the stimulatory effect of hpGRF-40. This suggested that GRF induces SRIF release by a mechanism which does not involve DA. To determine the possibility that DA and GRF may share a common pathway to stimulate SRIF release, ME fragments were simultaneously exposed to submaximal concentrations of both DA (0.6 µM) and hpGRF-40 (10^{-12}M). By themselves each of these doses had little effect on SRIF release. When added together a marked stimulation was noted. The results suggest that GRF may be physiologically involved in the regulation of SRIF release. Stimulation of SRIF release may be a mechanism by which GRF exerts an ultrashort feedback to inhibit GH release. Supported by NIH Grant AM-10073.

241.4 SECRETIN ELEVATES CYCLIC AMP LEVEL OF RAT BRAIN SLICES. R.T. Fremeau, Jr., L. Korman* and T.W. Moody. Dept. of Biochem., The George Washington Univ. Med. Ctr., Washington, D.C. 20037, and Dept. of Gastroenterology/Hepatology, VA Med. Ctr., Washington, D.C. 20422.

Secretin, a 27-amino acid peptide hormone isolated initially from acid extracts of the duodenum, is biologically active in the mammalian central nervous system (CNS). Intraventricular injection of secretin in rats produces alterations in dopamine metabolism, a reduction in prolactin secretion and reductions in both open field activity and novel item approach. In the rat superior cervical ganglion, secretin produces an acute dose-dependent increase in tyrosine hydroxylase activity, probably by elevating ganglionic cyclic AMP (cAMP) levels (Zigmond et al., Society for Neuroscience, 9: Abstract #9.12, 1983). Endogenous secretin-like immunoreactivity has been detected in mammalian brain extracts (O'Donohue et al., PNAS USA, 78: 5221, 1981) and receptors for secretin have been detected in rat brain (Fremeau et al., J. Neurosci., 3: 1620, 1983). Secretin receptors on neuroblastoma cell membranes couple with and stimulate adenylate cyclase (Roth et al., J. Neurochem., 42: 1145, 1984). Previously, Propst et al. (J. Neurochem., 32: 1495, 1979) found that secr-(5-27), a carboxy-terminal fragment of secretin, inhibited the secretin-stimulated accumulation of cAMP by neuroblastoma x glioma hybrid cells. We therefore decided to test whether secr-(5-27) could function as a secretin receptor antagonist in the CNS.

Secretin produced a dose-dependent increase in the cAMP content of rat brain frontal cortex slices when incubated with 10mM theophylline. Half-maximal increases occurred with a secretin concentration of 1µM which increased the cAMP level from 21.8 $\pm$ 4.3($\bar{x} \pm$ SD; n=6) pmols/mg protein to a secretin-stimulated value of 53.7 $\pm$ 6.1 ($\bar{x} \pm$ SD; n=6). In contrast to the 2.5x elevation in cAMP content produced by 1µM secretin, 1µM VIP produced 6-8x elevations. Preincubation with 25µM secr-(5-27) prior to the addition of 1µM secretin or 1µM VIP completely blocked the secretin-stimulated increase but had no effect on the VIP-stimulated increase in cAMP content of frontal cortex slices. Receptor binding studies indicated that secr-(5-27) inhibited ^{125}I-secretin binding to rat brain membranes with high affinity (K_i=400nM).

These data indicate that secretin stimulates cAMP production in rat brain slices and that secr-(5-27) may function as a central secretin antagonist.

241.5 EFFECT OF THE MICROINJECTION OF SUBSTANCE P (SP) ANTAGONIST [D-Pro^2,D-Trp^{7,9}]-SP INTO THE LATERAL HYPOTHALAMUS (LH) ON STRIATAL DOPAMINE METABOLISM. M. R. Melis and K. Gale. Dept. Pharmacol., Georgetown Univ., Schools of Med. & Dent., Washington, DC 20007.

We have recently reported that the intranigral injection of [D-Pro^2,D-Trp^{7,9}]-SP at a dose (20 ug) which does not modify the kinetic properties of striatal tyrosine hydroxylase (TH), prevented the activation of striatal TH and attenuated the increase in striatal homovanillic acid (HVA) levels induced by haloperidol (N.S. Arch. Pharm., in press) providing further evidence for an excitatory role of SP in the substantia nigra (SN). In support of this we found that the intranigral application of the SP agonist [pGlu^5, MePhe^8, MeGly^9]-SP$^{5-11}$ (20 ug) increased striatal HVA by 53%. However, paradoxically, the intranigral injection of 20 ug of [D-Pro^2,D-Trp^{7,9}]-SP by itself also increased striatal HVA by 51%. Experiments were undertaken to clarify this apparent contradictory effect. We report now that the microinjection of as little as 1 ug of [D-Pro^2,D-Trp^{7,9}]-SP into the LH, rostromedial to SN, induced a 60% increase in striatal HVA; in addition, a similar increase occurred in olfactory tubercle and nucleus accumbens. In contrast to the SN injections, LH injections (1 or 20 ug) did not prevent the haloperidol-induced activation of striatal TH. To see if the increase in HVA levels after intranigral application of [D-Pro^2, D-Trp^{7,9}]-SP was due to a diffusion into the LH, the SP agonist [pGlu^5, MePhe^8, MeGly^9]-SP$^{5-11}$ was microinjected into the LH of rats receiving the SP antagonist into SN. Interestingly, in the presence of the SP agonist in the LH,which, per se, did not induce any change in HVA levels, intranigral injection of [D-Pro^2,D-Trp^{7,9}]-SP significantly decreased striatal HVA levels. The present results indicate that at least two separable mechanisms exist by which [D-Pro^2,D-Trp^{7,9}]-SP can influence striatal dopamine metabolism: 1) a mechanism which involves the SN, whereby the SP agonist can increase and the SP antagonist can decrease striatal DA metabolism, respectively; and 2) a mechanism which involves the LH, whereby blockade of SP activity can cause an increase in DA metabolism, not restricted to the striatum.

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241.6 CENTRAL CONCENTRATIONS OF α-MSH AND CRF: EFFECTS OF FEVER AND HYPERTHERMIA. M. Holdeman*, O. Khorram*, W.K. Samson and J.M. Lipton. Physiology and Anesthesiology Departments, Southwestern Medical School, UTHSCD, Dallas, TX 75235

The concentration of α-MSH within the septum increases during fever and septal injections of α-MSH are antipyretic. CRF, when injected ICV, is also antipyretic. Using sensitive radioimmunoassays of microdissected tissue extracts, we have established the presence of immunoreactive α-MSH (ir MSH) and CRF (ir CRF) within discrete CNS sites of the rabbit. Leukocytic pyrogen-induced fever (avg. Δ °C=1.78) and hyperthermia due to heat-stress (avg. Δ °C=2.31) did not alter concentrations of ir MSH or ir CRF in tissue extracted from midbrain central grey or preoptic/anterior hypothalamic regions. However, significantly greater levels of ir MSH were detected in septal extracts of febrile rabbits (0.33 ± 0.04 pg/μg protein,n=10) than in septal extracts from afebrile controls (0.12 ± 0.02 pg/μg protein,n=8) or heat-stressed animals (0.11 ± 0.02 pg/μg protein,n=12). A significant decrease in ir CRF was detected in paraventricular nucleus extracts from febrile animals (6.24 ± 0.40 pg/μg protein,n=10) compared to extracts from afebrile controls (13.12 ± 0.64 pg/μg protein,n=8) or heat-stressed rabbits (12.93 ± 1.49 pg/μg protein,n=9). Our results support the hypothesis that these central peptides have a role in temperature control during fever. Since no changes were detected in extracts from hyperthermic rabbits, it appears that changes in concentration of these neuropeptides in particular brain regions are specific to the febrile state and are not caused by simple elevation of body temperature. Neuropeptide activity may be induced by the presence of endogenous pyrogen (interleukin 1), or some other mediator of the febrile response, as a protective measure against acute temperature elevation during infection. The decrease in paraventricular CRF may reflect transport from the CRF-producing cell bodies of the PVN to a region yet to be determined. (Supported by NINCDS Grant 10046)

241.7 IONTOPHORETIC APPLICATION OF HUMAN PANCREATIC TUMOR-DERIVED GROWTH HORMONE RELEASING FACTOR: ELECTROPHYSIOLOGICAL INVESTIGATION OF NEURONAL SENSITIVITY IN RAT FOREBRAIN. M.J. Twery* and R.L. Moss, Dept. Physiol., UT Hlth. Sci. Ctr., Dallas, TX 75235.

Human pancreatic tumor-derived growth hormone releasing factor (hpGRF) is a potent growth hormone (GH) secretagogue with biological and chemical properties which closely resemble those of the putative hypothalamic GRF. The present investigation studied the action of hpGRF on the extracellular, single cell activity of neurons localized in rat forebrain, the distribution of hpGRF-sensitive neurons, and the electrophysiological response of these neurons to somatostatin (SS), a GH release inhibiting factor. Experiments were performed on male Long Evans rats (250-400g) anesthetized with urethane (1.2 mg/kg, IP). A multi-barrelled glass microelectrode was used for extracellular recording of single units (center barrel, 4M NaCl, 3-9MΩ) and iontophoretic application of chemicals.

Of 109 cells from which action potentials were recorded in experiments with 13 rats, the membrane sensitivity to both hpGRF and SS was determined in 67 cells, the effect of only hpGRF was tested in 13 cells, and the remaining 29 cells were excluded from the analysis due to insufficient data or questionable localization. hpGRF altered the single unit activity of over half the cells to which the peptide was applied (43 of 80). Of the neurons sensitive to hpGRF, the predominant response was a decrease in firing rate (33 of 43). Neurons inhibited by hpGRF were found in cortex (9 of 13), thalamus (9 of 34), and hypothalamus (15 of 33). hpGRF increased the firing rate of few neurons in the thalamus (N=6), and hypothalamus (N=4). Of the cells tested with hpGRF and SS; 52% of the 27 cells inhibited by hpGRF were also inhibited by SS, 37% of the 8 cells stimulated by hpGRF were also stimulated by SS, and in 59% of the 32 cells whose firing rate was unaffected by hpGRF, SS also produced no change. The results clearly demonstrate that hpGRF is able to alter the excitability of neurons in several brain regions. The distribution of GRF-sensitive neurons suggests that hypothalamic GRF, in addition to its well documented role in the regulation of pituitary GH secretion, may subserve a broad neuromodulatory function in the CNS.

We thank Dr. Wylie Vale (Salk Institute) for providing the hpGRF used in this study. Supported by NIH grant NS10434.

241.8 BRAIN AND SPINAL CORD NEUROPEPTIDES AFTER SCIATIC NERVE SECTION OR CHRONIC ARTHRITIS. A.E.Panerai,P.Sacerdote*,V.Locatelli*,P.Mantegazza*.Dept.Pharmacology,School of Medicine, Univ.of Milano,Milano,20129,Italy.

We evaluated B-endorphin(BE),Met-enkephalin(ME),Somatostatin,Substance P in the brain areas and different sections of the spinal cord in rats that underwent either monolateral section of the sciatic nerve,or Freund adjuvant induced chronic arthritis. The aim of the study was to determine if and how did peripheral supposedly painful lesions of different origin influence the central nervous system concentrations of neuropeptides involved in the modulation of pain. Peptides were evaluated by radioimmunoassay and immunohystochemistry. In rats that underwent the section of the sciatic nerve,measurements of neuropeptides were done twentyfour hours and seven,fourteen,twentyone and thirtyfive days after the lesion. In rats with arthritis,neuropeptides were evaluated seven,fourteen,twentyone, and thirtyfive days after the induction of arthritis by injection of complete Freund adjuvant at the base of the tail. In the case of sciatic nerve section, since this was always made on the right side,left and right brain areas were evaluated separately, while this precaution was not extended to chronic arthritis,since this was always bilateral. BE concentrations decreased bilaterally 20/80% in brain areas at different times since section of the sciatic nerve or induction of arthtritis and showed a trend toward normal values in arthritic rats after day twentyone,when the degree of arthritis decreases. ME increased significantly in the lumbosacral spinal cord at twentyone and thirtyfive days since the section of the sciatic nerve,and twentyone days since the induction of arthritis. Of the other peptides,only substace P showed a trend toward an increse in the lumbosacral spinal cord,in both experimental models. Pharmacological treatments were also attempted,in order to normalize the concentrations of neuropeptides. In conclusion,our data indicate a prompt and severe involvement of brain neuropeptides in response to"peripheral"modificatio ns of inputs probably related to painful stimuli.

241.9 SUBSTANCE K EXCITES SUBSTANTIA NIGRA NEURONS IN RAT. R.B. Innis, R. Andrade and G.K. Aghajanian. Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508.

Substance K (SK) is a recently discovered neuropeptide, which has significant amino acid homology to substance P (SP) and is encoded in a region adjacent to SP in the preprotachykinin gene(1). The discovery of this new mammalian tachykinin may explain the apparent lack of electrophysiologic activity in brain areas which are believed to have rich SP innervation. For example, the substantia nigra (S.N.) is densely innervated with SP-like immunoreactive nerve terminals. However, we have found that the microiontophoretic administration of SP in chloral hydrate anesthetized rat is without effect on the vast majority of S.N. dopaminergic and non-dopaminergic neurons. Similarly, no clear effect of SP on glutamate, dopamine, GABA, neurotensin, or serotonin reponses has been observed.

In contrast to the results with SP, we have found that microinontophoretically applied SK excites greater than 25% of dopaminergic and non-dopaminergic neurons in rat S.N. Kassinin, a peptide very similar to SK also excites S.N. neurons. These results are consistent with receptor autoradiographic studies which show a lack of SP receptors(2) but an abundance of SK receptors in the S.N. (J.Maggio, personal communication). In this case, the specificity of peptide action is determined by the specificity of the post-synaptic receptor.

Neurotensin provides a further example. The S.N. receives innervation with this peptide and neurotensin receptors are present on dopaminergic neurons, which are located in the zona compacta of S.N.(3). Consistent with this, we have found that microiontophoretically applied neurotensin excites many of the dopaminergic but none of the non-dopaminergic neurons of the SN.

In summary, we have found that SK excites dopaminergic and non-dopminergic neurons in the S.N. In contrast, neurotensin excites exclusively the dopaminergic neurons, and SP is largely without any effect. These physiological results are consistent with receptor autoradiographic studies which show in the S.N. a lack of SP receptors, dense SK receptors, and neurotensin receptors specifically on dopaminergic neurons.

1. H. Nawa et al. Nature 306: 32, 1983.
2. R. Quirion et al. Nature 303: 714, 1983.
3. J. Palacios and M. Kuhar Nature 294: 587, 1981.

241.10 THE CHOLECYSTOKININ AGONIST CERULETIDE STIMULATES GROWTH HORMONE AND PROLACTIN RELEASE IN RHESUS MONKEYS. G.R Heninger, R.B.Innis, D.S. Charney*, and B.S. Bunney. Dept. of Psychiatry, Yale Med. Sch. New Haven, CT 06508.

Cholecystokinin (CCK) and gastrin (GAS) are classical gastrointestinal polypeptide hormones which have been shown to have important effects on the release of anterior pituitary hormones and other CNS functions. CCK and GAS share a common 5-amino acid sequence at the carboxy terminus. In order to assess their effects on anterior pituitary function in primates, the CCK agonist ceruletide and the GAS agonist pentagastrin were administered to rhesus monkeys with and without prior administration of the centrally active CCK antagonist proglumide.

METHODS: Seven adult male rhesus monkeys (7-10 kg) were chair adapted and had repeated saphenous vein catheterizations. A 2 hr. adaptation period was followed by repeated blood sampling beginning 30 min. prior to and continuing 90 min. after the 15 min. IV infusion of peptides. Plasma was assayed for growth hormone (GH) and prolactin (PRL) with standard RIA methods.

RESULTS: At the 3 doses used (0.5, 2, and 8 ug/kg), ceruletide produced a mean peak increase from baseline in GH of 5, 6, and 10 ng/ml and in PRL of 10, 17, and 37 ng/ml, respectively. At the 8 ug/kg dose every monkey vomited in the middle of the infusion. Pentagastrin produced no change in GH or PRL levels and no vomiting at any of the 4 doses studied (1, 2, 4, and 8 ug/kg). Proglumide at 40 mg/kg produced a 29% and 63% and at 120 mg/kg a 62% and 60% inhibition of the 2 ug/kg ceruletide stimulation of GH and PRL, respectively. Proglumide had no effect when given alone.

DISCUSSION: The failure of .3 ug/kg of 1M ceruletide to alter GH and PRL in humans (Lal, S. Prog. Neuro-Psychopharmacol. & Biol. Psychiat. 7:537, 1983) may be due to the relatively lower dose. The specificity of ceruletide's action is supported by the dose-related increases in GH and PRL, the ability of the centrally active antagonist proglumide to block the increases, and by the inability of the closely related peptide pentagastrin to produce the hormonal respose. Cerulitide stimulation of anterior pituitary hormonal function may be a useful method to assess the CCK modulation of neuroendocrine function in a variety of medical, neurologic and psychiatric conditions.

241.11 INTRATHECAL (I.T.) INJECTION OF BOMBESIN AND RAT CRF INHIBITS GASTRIC ACID SECRETION IN RATS. D. Hamel*, and Y. Tache. Center for Ulcer Research and Education (CURE), University of California, School of Medicine, VA Wadsworth, Los Angeles, CA.

Intracisternal injection of bombesin, corticotropin-releasing factor (CRF), calcitonin-gene-related peptide (CGRP), and dynorphin dose dependently inhibited and thyrotropin-releasing hormone (TRH) or RX 77368 a stable TRH analog, stimulated gastric acid secretion in rats. Based on the supraspinal and spinal localization of these peptides, studies were undertaken to investigate if rat CRF (rCRF), rat CGRP (rCGRP), TRH and dynorphin delivered into the spinal subarachroid space at the lumbar L2 level will also influence gastric secretion.

In 24h-fasted male Sprague-Dawley rats (220-260g) under ether anesthesia, 8.5 cm length polyethylene cateters were inserted through a slit made in the cisternal membrane, and peptides or 0.1% bovine serum albumin were injected with 5ul volume followed by 5ul vehicle to wash the catheter. The pylorus was then ligated and 2h later, conscious rats were decapitated for collection of gastric secretion. RX 77368 (3,3'-dimethyl)^{3}TRH (0.01-1ug) injected i.t. did not significantly modified gastric secretion whereas intracisternal injection markedly elevated gastric acid output. Bombesin (0.05-1ug) and rCRF (0.01-10ug) given i.t. induced a dose-dependent inhibition of gastric secretion . Highest doses of bombesin and rCRF reduced gastric acid output by 81-86%. CRF action was not reversed by adrenalectomy. By contrast dynorphin or rCGRP (0.1-10mg) given i.t. did not modify gastric acid secretion. These results suggest that bombesin and rCRF unlike rCGRP have supraspinal and spinal sites of action to inhibit gastric acid secretion. The stimulatory action of TRH appears to require direct action on specific supraspinal structures.

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241.12 ACTIVATION OF BENZODIAZEPINE RECEPTORS SUPPRESSES CHOLECYSTOKININ-INDUCED EXCITATION OF HIPPOCAMPAL PYRAMIDAL NEURONS: A MICROIONTOPHORETIC STUDY IN THE RAT. J. Bradwejn* and C. de Montigny, Neuroscience Research Center, Université de Montréal, Montréal, Canada, H3C 3J7.

Male Sprague-Dawley rats (225-300g) were anesthetized with urethane (1.25g/kg, i.p.). Five-barrelled micropipettes were used for extracellular unitary recording of CA_3 dorsal hippocampus pyramidal neurons. The following solutions were used for microiontophoresis: sulphated cholecystokinin octapeptide [CCK-8S] (10 µM in NaCl 0.2 M, PH: 5; Squibb), acetylcholine.HCl [ACh](20 mM in NaCl 0.2 M, PH: 4; Calbiochem), flurazepam.HCl [FLU] (20 mM, PH: 4; Hoffman-Laroche), chlordiazepoxide.HCl [CDP] (10 mM in NaCl 0.02 M, PH: 3; Hoffman-Laroche) and methionine-enkephaline [mENK] (0.5 mM in NaCl 0.2 M with BSA 0.01%, PH: 4.6; Sigma).

The i.v. administration of high doses of FLU, lorazepam [LOR] (Wyeth) and diazepam [DZ] (Hoffman-Laroche) completely abolished the CCK-8S-induced activation in a selective manner as mENK- and ACh-induced activations were not affected. The ED_{50} of DZ and LOR obtained from dose-response curves for suppressing CCK-8S-induced activation were 100 µg/kg and 20 µg/kg, respectively. Microiontophoretic applications of FLU and CDP reversed the CCK-8S-induced activation but not that induced by mENK or ACh. Both the effects of i.v. LOR and microiontophoretic FLU were completely prevented or reversed by RO 15-1788 (3.5 mg/kg, i.v.).

PK 8165, a non-benzodiazepine [BZD] quinoleine derivative, is an anxiolytic agent which binds to BZD receptors. Low i.v. doses and microiontophoretic applications of PK 8165 also suppressed selectively the CCK-8S-induced activation (ED_{50} = 250 µg/kg, i.v.). This effect of PK 8165 was antagonized by RO 15-1788 (3.5 mg/kg, i.v.). High i.v. doses of PK 8165 failed to suppress the CCK-8S-induced activation but antagonized the effect of LOR on the activation by CCK-8S.

These results show that activation of BZD receptors suppresses selectively the excitation of hippocampal pyramidal neurons by CCK-8S and that, in keeping with biochemical findings, PK 8165 is a mixed agonist-antagonist of BZD receptors. The ED_{50}'s obtained with LOR, DZ and PK 8165 fall exactly in the range of their anxiolytic doses in humans, thus suggesting that this neuropharmacological phenomenon might be involved in mediating the anxiolytic effect of drugs activating the BZD receptors.

242.1 THE FIBROUS CYTOPATHOLOGY OF DEGENERATING NEURONS IN ALZHEIMER'S DISEASE: AN ULTRASTRUCTURAL REAPPRAISAL. D.J. Selkoe. Harvard Med. School, Mailman Research Center, McLean Hospital, Belmont, MA 02178.

Classical descriptions of the cytopathology of human neuronal degeneration during aging and in dementia of the Alzheimer type (AD) include the accumulation of abnormal cytoplasmic fibers in neuronal cell bodies [as neurofibrillary tangles (NFT)] and in altered neurites (as neuritic plaques). Ultrastructural analyses during the past two decades have concluded that the principal pathological fibers in these lesions are pairs of intermediate (~10 nm) filaments twisted into double helices with a half-periodicity of ~80 nm. However, reports of NFT composed of straight 15 nm filaments in certain cases of AD have recently been published. We have examined formalin-fixed hippocampal tissues obtained postmortem from 3 cases of AD, including one with familial (autosomal dominant) AD, by transmission electron microscopy (EM). In all cases, light microscopy demonstrated abundant NFT and neuritic plaques showing classical morphology and argyrophilia. However, EM revealed that both neuronal perikarya and neurites displayed a complex mixture of abnormal cytoplasmic fibers, including: (1) straight (non-helical) filaments with a spectrum of diameters between 10 and 20 nm, most commonly 15 nm; (2) paired helical filaments (PHF) varying between 20 and 24 nm in maximal diameter and having half-periods of 75-85 nm; (3) 15-20 nm filaments which were not entirely straight but appeared to show subtle periodic constrictions. Random mixtures of 15-20 nm straight filaments and PHF occurred within the same fibrous bundle in certain neuronal perikarya or neurites; this was particularly common in the autosomal dominant AD case. We also observed apparently extracellular bundles of straight 15-20 nm filaments in the latter case. Like PHF, 15-20 nm straight filaments were present in the insoluble fraction of AD cortex following SDS extraction. In a case of the much less common presenile dementia, Pick's disease, EM of the characteristic intraneuronal Pick bodies also showed a spectrum of abnormal fibers, principally 12-20 nm straight filaments, with occasional typical (~80 nm half-periodicity) or atypical (>100 nm half-periodicity) PHF found in the same neuron. We conclude that the fibrous cytopathology of neurons in AD is more complex and heterogeneous than generally stated and includes the accumulation of straight cytoplasmic fibers with a spectrum of diameters from 10 to 20 nm as well as PHF. Molecular models and hypotheses for the reorganization of the fibrous neuronal cytoskeleton in AD will need to take this complexity into account.

242.2 CHOLINE ACETYLTRANSFERASE IMMUNOREACTIVITY IN HEREDITARY CANINE SPINAL MUSCULAR ATROPHY. L. C. Cork*, R. J. Altschuler*, J. W. Griffin, D. L. Price and B. H. Wainer. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Immunocytochemical (peroxidase/antiperoxidase) methods relying upon a monoclonal antibody directed against choline acetyltransferase (ChAT) were used to examine the properties of motor neurons in dogs with Hereditary Canine Spinal Muscular Atrophy (HCSMA), an autosomal dominant motor neuron disease. HCSMA has three phenotypes: accelerated, intermediate, and chronic. Dogs with accelerated disease are homozygous for the HCSMA trait and develop quadraparesis by four months of age. Heterozygous HCSMA dogs have intermediate or chronic disease. Dogs with intermediate disease develop marked paresis between two and three years of age; those with chronic disease have denervation atrophy in proximal muscle groups but are not severely compromised at seven years of age. Dogs with HCSMA show large, proximal, neurofilament-filled internodal axonal swellings, and gel fluorographic studies disclose impairment of transport of neurofilament polypeptides and, to a lesser degree, tubulin and actin. Using a monoclonal antibody against bovine ChAT, we compared ChAT immunoreactivity in one intermediate and two chronic HCSMA dogs (3 and 7 years old) to that of control dogs. Motor neurons showed ChAT immunoreactivity in control, chronic, and some, but not all, motor neurons in intermediate HCSMA dogs. However, some large ventral horn neurons in dogs with intermediate HCSMA did not appear to express ChAT immunoreactivity, although ChAT-immunoreactive fibers appeared to terminate on their surface. Unlike controls, the surface of many ChAT-immunoreactive neurons in HCSMA dogs contained large ChAT-immunoreactive fiber terminals. Axonal swellings in dogs with intermediate HCSMA showed ChAT immunoreactivity; chronic HCSMA dogs had virtually no axonal swellings. These observations suggest that ChAT may be retained in axonal swellings in dogs with intermediate HCSMA and that all motor neurons in these dogs may not express ChAT activity. Our results raise the possibility that abnormalities in the synthesis and/or transport of ChAT may have functional correlates in HCSMA and, possibly, in its human counterpart, familial motor neuron disease.

242.3 NEUROPEPTIDE Y-CONTAINING CELLS IN HUNTINGTON'S CHOREA, AND IN THE KAINIC ACID LESIONED STRIATUM AS AN ANIMAL MODEL OF THE DISEASE. Y. S. Allen*, G. Cole*, T.J. Crow+, B. Brownell++ and J.M. Polak. Dept. of Histochemistry, Royal Postgraduate Medical School, London, U.K., +Division of Psychiatry, Clinical Research Centre, Harrow, Middlesex and ++Department of Neuropathology, Frenchay Hospital, Bristol, U.K.

Huntington's Chorea is an inherited degenerative disorder of the central nervous system which manifests itself in middle age with the symptoms of dementia, neuroendocrine disturbance and severe dyskinesia. Neuropathological examination has revealed substantial cell loss from many brain regions, but most markedly the caudate nucleus. Neurochemical analyses have described a generalised depletion of enzyme markers for the neurotransmitters contained within cells of the striatum while the nigrostriatal dopaminergic innervation remains unaltered. It is commonly agreed that these morphological and biochemical changes are well mimicked by the lesioning of the animal striatum with kainic acid, a neurotoxin selective for cell bodies (Coyle, J.T. and Schwarcz, R., Nature (Lond.), 263: 244, 1976; McGeer, E.G. and McGeer, P.L. Nature (Lond.), 263: 517, 1976).

Recently, a number of neuropeptides (including neuropeptide Y) have been described to be contained within cells of the striatum. Furthermore, reported radioimmunoassay results would indicate that neuronal elements containing neuropeptide Y are preserved in Huntington's Chorea (Emson, P.C., Rosser, M.N. and Hunt, S.P., Peptides and Neurological Disease, Cambridge, England, Aug. 1983). Indeed, our immunocytochemical investigations on thick, vibratome sections of Huntington's tissue would support this view. However, our studies show that these cells do not survive kainate lesioning of the rat striatum, a treatment which otherwise mimicked the changes seen in Huntington's Chorea. These observations have important implications both for indicating the selectivity of cell loss from the striatum inherent in the disease process, and for highlighting the more subtle, neurochemical changes the kainate model of Huntington's Chorea cannot duplicate.

242.4 EFFECTS OF QUINOLINIC ACID ON STRIATAL GLUCOSE CONSUMPTION OF RATS. M.P.Heyes* and E.S.Garnett* (SPON: J.Seggie). Section of Radiology and Nuclear Medicine, McMaster University Medical Centre, Hamilton, Ontario, Canada, L8N3Z5.

Garnett et al (J. Neurosci: In Press) using positron emission tomography demonstrated that patients in the very early stages of juvenile-onset Huntington's disease (HD) have depressed striatal glucose consumption (CMR_{gluc}). These patients have little or no striatal atrophy, assessed by CAT scan, and present with psychiatric disturbances rather than movement disorder. The decreased striatal CMR_{gluc} may reflect changes in neuron electrical activity due to imbalances in neurotransmitter action. Certain anatomical and neurochemical characteristics of HD, including depressed striatal CMR_{gluc}, can be replicated in rats by injecting kainic acid (KA) directly into the striatum. KA does not occur naturally in the brain but because it is a structural analog of glutamate it has been postulated that excessive glutamatergic activity is responsible for the striatal cell death of HD. It has recently been shown that when quinolinic acid (QA) is injected into the striatum it has neurotoxic properties similar to those of KA (Schwarcz et al Science 219: 316, 1983). QA is a natural product of tryptophan metabolism. It is possible therefore that excessive quantities of QA might be produced in HD. We have studied the effects of QA on striatal CMR_{gluc} measured by the [^{14}C]-2-deoxyglucose (2-DG) technique. An intrastriatal injection of 250 nmoles of QA (dissolved in 1.8μL of saline infused over a 25 min period) caused an immediate increase in CMR_{gluc} in the ipsilateral striatum. Four days later striatal CMR_{gluc} was reduced and the striatum itself was slightly swollen. Our observations show that QA is similar to KA with respect to its effects on striatal CMR_{gluc}. We suggest that the reduction of CMR_{gluc} in HD may be a consequence of an increased amount of QA or increased sensitivity of striatal neurons to QA.

MPH is a recipient of a post-doctoral fellowship from the Huntington Society of Canada.

242.5 METABOLISM OF THYROTROPIN-RELEASING HORMONE (pGlu-His-ProNH$_2$, TRH) IN THE CEREBROSPINAL FLUID (CSF) OF PATIENTS WITH NEUROLOGIC DISORDERS. T. FREDERICK,* A. JAYARAMAN, R.M. EDWARDS,* AND C. PRASAD. (SPON:L.HAPPEL). Depts. of Neurology, Medicine and Biochemistry, Louisiana State University School of Medicine, New Orleans, LA. 70112.

TRH has been suggested to be beneficial in the neurological recovery in experimental spinal cord trauma, stroke and ALS. To understand the potential role of TRH in these disorders, we have studied TRH metabolism in CSF of 31 consecutive patients admitted with neurological disorders. CSF was incubated with [^{3}H-Pro]-TRH under optimal conditions (50 mM Tris-acetate buffer, pH 6.8, 37C). TLC analyses show that TRH is metabolized to proline, acid TRH (A-TRH), and cyclo (His-Pro) (cHP). The cHP/(proline+A-TRH) ratio was greater than five, sugesting that pGlu-peptidase to be the major pathway in human CSF. There was no apparent correlation between the individual neurological diseases and the rate of formation of TRH metabolites. However, in acute destructive lesions within CNS or in acute exacerbation of chronic neurolgical conditions, the metabolism of TRH through the amidase pathway (A-TRH+proline) was increased, with a compensatory decrease of the metabolism through the peptidase pathway.

Neurologic disorder	Total TRH metabolized	% OF TRH metabolized to	
		A-TRH+Pro	cHP
Acute (13)	74.8±28.4	28.7±6.2	71.2±6.1
Chronic (18)	49.0±13.9	12.0±2.7	88.0±2.7
p-value vs. acute	>0.20	< 0.02	<0.01

*pmols/min/mg/protein (mean+SEM)

The altered TRH-metabolic pattern may be related to tissue damage, tissue repair or due to stress associated with these conditions.

242.6 THE NEW APPROACH FOR CLASSIFICATION OF SCHIZOPHRENIA USING POSITRON CT (PET). H.Kishimoto, H.Kuwabara*, T.Miyauchi*, S.Oono*, Y.Nomura*, O.Takazu*, T.Ishii*, S.Yokoi*and M.Iio*. (SPON: T.Takenaka) Department of Psychiatry, Yokohama City University, Yokohama 232 and Nakano National Hospital, Nakano, Tokyo 165, JAPAN.

The concept of schizophrenia was established by Bleuler in 1911. The schizophrenic patients really exists all over the world, however, most of the biological bases for schizophrenia are still unknown. The positron emission tomography is an important new instrument for understanding the function of the working brain. It is expected this instrument will provide new biological information leading to a better understanding of mental illness especially schizophrenia and affective disorders. In this presentation we would like to describe the finding of experiments using PET in a new classification of schizophrenia.

Twelve schizophrenic patients (8 men, 4 women, mean age 40) who were out and inpatients at the Yokohama University Hospital, and six control subjects (men,mean age 41) who agreed to cooperate with this test participated in this study. Diagnoses were based on DSM-III criteria. The patients' histories of illness were from seven to twenty-six years in length. The subjects were laid down on the CT bed in a darkened room with eyes closed and given 10mCi $^{11}CO_2$ with oxygen by inhalation or 20mCi ^{11}C-glucose by per os. After the ^{11}C-tracer administration, two to six horirontal brain scans parallel to the OM line were done.

The major findings of this study were as follows : There was no significant difference between the counts (25 pixels counts/total counts) of control subjects and schizophrenic patients in the area of the tempolar cortex, the occipital cortex, striatum and thalamus; however, statistical significance was found in the area of the frontal cortex by $^{11}CO_2$ ($p<0.05$) and ^{11}C-glucose ($p<0.05$) administration. In detail, there are at least two types of PET image (I) hypofrontal schizophrenic patients (number 8) and (II) normal frontal schizophrenic patients (number 4). When hypofrontal patients were taken together, the statistical significance in the area of the frontal cortex became remarkable in both $^{11}CO_2$ ($p<0.001$) and ^{11}C-glucose ($p<0.001$) administration, however, normal frontal patients had no statistical significance compared to controls. The type of clinical picture for hypofrontal patient is similar to Residual Type and that for normal frontal patient is similar to Undifferentiated Type by DSM-III criteria. Supported by Grant 83-10-11 (NCNMMD) of the Ministry of Health and Welfare, Japan.

242.7 NMR STUDIES OF SCHIZOPHRENIA. R.C. Smith, M. Calderon, R. Baumgartner, G.K. Ravichandran, J.C. Schoolar. Biological Psychiatry, Texas Research Institute of Mental Sciences, and Houston Imaging Center, Houston, Texas 77030.

Several studies of our own and other groups have demonstrated morphological abnormalities in the brains of schizophrenic patients using quantitative measurements from CT scans and related techniques. Nuclear Magnetic Resonance Scans (NMR) provide much greater detail of brain anatomical structure and several different imaging modes which provide information relevant to properties of the atomic nuclears, which may be used to characterize brain tissues, such as proton density (p) and T_1 and T_2 relaxation times. We report the first studies of quantitative measurements in the brains of schizophrenic patients utilizing NMR. Twenty-five schizophrenic and 20 controls were scanned with a .3 Tesla NMR scanner using several scanning modes (Spin-Echo (SE)-30, Inversion Recovery (IR)-30, and SE-120) which highlighted the p, T_1 and T_2 properties, respectively. The greater detail available on NMR scans allowed measurement of many structures not easily visualized on CT scans. Quantitative analysis of linear and area measures of image intensities revealed: (a) schizophrenics had higher image intensities of white matter and grey matter in the right and left anterior region of coronal slices, scanned in the IR30 mode; (b) schizophrenics had lower image intensity of the left putamen-globus pallidus than controls; (c) there was a strong trend for larger bicaudate widths, and reversal of normal left vs right areas asymmetrical of the later ventricles in schizophrenics than controls; (d) there were significant correlations of quantitative measures of psychopathology reflecting of "positive" and "negative" schizophrenic symptoms with brain morphology measures. The differences in densities in the IR30 mode between schizophrenic and controls are consistent with the interpretation that the tissue in some areas of the brains of schizophrenics may have longer T_1 relaxation times than normal controls.

242.8 QUANTITATIVE ANALYSIS OF REGIONAL BRAIN VOLUMES FROM MEDICAL IMAGE DATA. D.S. Schlusselberg, W.K. Smith, B.G. Culter*, R.B. Parkey* and D.J. Woodward, Depts. of Cell Biology and Radiology, Univ. Texas Health Science Ctr., Dallas, Texas 75235.

A computer system developed for three-dimensional reconstruction of serially sectioned material has been adapted for analysis of data acquired from medical imaging modalities such as Computed Tomography (CT) and Magnetic Resonance Imaging (MRI). The ability to quantitate both normal and pathological variations in specific brain regions in humans is made possible by the general purpose nature of the computer graphics system that is used.

CT provides serial transverse sections of human brain with high spatial resolution, but with contrast limited to gross changes in tissue density (i.e. bone, water, fat). Spatial resolution is usually greater in the plane of sectioning than between sections. MRI can generate serial section images in any orthogonal plane, and is capable of true three-dimensional data acquisition with equal spatial resolution in all three dimensions. Contrast between grey and white matter allows assesment of cortical grey matter and subcortical nuclear regions. High Field MRI will provide images of metabolic activity within the brain.

Individual sections from these imaging modalities are displayed on a raster scan CRT with graphics overlay. Boundaries of objects of interest are either manually traced or generated automatically by a high-speed edge detection algorithm. A sophisticated hierarchical database is used to store perimeters and image density information within the boundaries. Three-dimensional regions are selected by combining data from sequential sections, and this connectivity information is stored in the database. Quantitative information such as volume, surface area, long and short axes, and regional density distribution can be obtained for selected three-dimensional structures that have been defined in the database. Our view is that this flexible system for determining brain volumes will be useful for the study of cerebral hemispheric and regional brain asymmetries, and monitoring the progression of metabolic and malignant disease.

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242.9 SELECTIVE LABELING OF ISCHEMIC BRAIN DAMAGE BY RADIOLABELED IONS. G.A. Dienel and W.A. Pulsinelli. Dept. Neurology, Cornell Univ. Med. Col. New York, N.Y. 10021.

Clinical diagnosis of human ischemic brain injury and assement of the size, location, and temporal progression of the injury could be improved if markers for lethally injured cells were available for positron-emission tomographic (PET) scanning of patients. Positron-emitting ions that mimic calcium, an ion known to be sequestered by dying tissue, could be used to locate and quantify ischemic injury. To identify radiochemicals that are preferentially concentrated in brain regions undergoing neuronal necrosis, we measured the regional concentrations of radionuclides after forebrain ischemia in the rat. Forebrain ischemia was produced by temporary (30 min) occlusion of the carotid arteries and permanent occlusion of the vertebral arteries. The animals were allowed to survive for 24h after restoration of carotid artery blood flow. At this time, the majority of the medium-sized neurons in the striatum are irreversibly damaged, whereas most of the neocortical neurons escape injury. At 24h survival, the radiochemicals were injected intravenously and allowed to circulate for 5 h. The brains were perfused to remove blood from the cephalic circulation, and radiochemical concentrations were determined in dissected regions or by autoradiography. In normal controls, autoradiographic analysis of the distribution patterns of some of these ions showed that TcO_4 and Ni were most uniformly distributed throughout the brain; PO_4 labeling was heterogeneous, with higher amounts of PO_4 in the outer layers of the neocortex, and in the periventricular tissue and choroid plexus. Pm was localized primarily in the choroid plexus, with very little penetration of the brain tissue. The permeability of the brain to these radiolabeled ions varied over a large range. The ions are listed in order of decreasing brain concentration, expressed as percent of the injected dose at 5 h after the injection (given in parentheses): Na(45%), PO_4 (4%), Ni(0.2%), and TcO_4 and Pm (0.01-0.05%). In postischemic animals, four radionuclides (Na, Ni, TcO_4, PO_4) were concentrated in the damaged striatum in amounts ranging from 1.4 to 5-fold greater than in the undamaged neocortex; labeling of the striatum by Pm was not significantly greater than cortex. Of the radionuclides tested to date, sodium, nickel, and technetium have positron-emitting isotopes which may be suitable for positron-emission scanning in humans.

242.10 PROTEIN PATTERNS IN VARIOUS HUMAN BRAIN TUMORS USING TWO-DIMENSIONAL GEL ELECTROPHORESIS. R.K. Narayan, W.E. Heydorn, G.J. Creed, P.L. Kornblith and D.M. Jacobowitz. Lab. of Clinical Science, NIMH and Surgical Neurology Branch, NINCDS, Bethesda, MD 20205.

Brain tumor samples from 50 patients including 24 astrocytomas, 9 meningiomas, 5 pituitary adenomas, 4 medulloblastomas, 2 craniopharyngiomas, 3 juvenile astrocytomas, 1 ependymoma, 1 schwannoma and 1 choroid plexus papilloma were studied using two-dimensional gel electrophoresis (2DE) with silver staining. In addition, 12 different samples of fresh-frozen normal human cerebral cortex, previously irradiated cortex and post-mortem cortex were analyzed using computerized densitometry. Tissue samples obtained at surgery were sectioned by alternating 300 μm thick sections with thin sections. The latter were stained and used to localize the area of interest for micropunch sampling from the frozen thick sections, thus establishing the histological correlates of the biochemical patterns. 130 consistent protein spots were noted on gels from normal human cerebral cortex with a MW range of 14.4 K to 100 K and a pI range of 4.7 to 7.0. There were significant quantitative differences between the three groups of "normal" human cortex, although the qualitative patterns were very similar from patient to patient. However, even more remarkable was the finding that histological tumor type was associated with its own fairly distinctive and reproducible protein pattern. For example, the astrocytomas demonstrated prominent albumin and glial fibrillary acidic protein (GFAP) spots with high but variable concentrations of neuron specific enolase (NSE). Meningiomas, on the other hand, did not contain any NSE or GFAP. So far, six of the major spots on these gels have been identified (albumin, actin, alpha and beta tubulin, GFAP and NSE). These findings indicate that biochemical diagnosis and grading of human brain tumors based on their protein fingerprints is a realistic possibility. Using just a few milligrams of tumor tissue, such a system could supplement data provided by histological studies and yield insights into brain tumor associated antigens.

242.11 BLOOD CONCENTRATION OF BRADYKININ IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS.
L.Germain ,J.Barabé and C.Galeano .(SPON:E. Ramon-Moliner) Department of Physiology and Pharmacology,Faculty of Medecine,University of Sherbrooke,Sherbrooke,Québec,Canada J1H 5N4

Experimental allergic encephalomyelitis (EAE) is a demye linating disease of the central nervous system (CNS)caused by an hypersensibility reaction to myelin basic protein. Among several inflammatory mediators that have been postulated to play a role in the modulation and / or control of this auto-immune process the kallikrein-kinins system has been underevaluated.However kinins have several pro-inflammatory action such as producing pain and vasodilatation, increasing vascular permeability and promoting the proliferation of lymphocytes.Beside conduction failure a major physiological alteration in EAE is the increase in blood-brain barrier permeability.We therefore manage to evaluate the possible role of kinins in EAE by measuring the blood concentration of bradykinin during EAE.

Methods:New-Zealand rabbit were inoculated with guina pigs spinal cord in complete Freund's adjuvant.The animals developed clinical signs of EAE and then under chloralose anesthesia arterial blood sampling was withdraw from caro tid .The blood samples were proceed to measure bradykinin level by radioimmunoassay.The animals were sacrificed and perfused with formalin .The CNS were harvested and prepared for histological examination at hypothalamic,midbrain,pontine,bulbar and spinal levels.

Results:In a first experiment 6 EAE and 4 Controls animals were examinated.In the EAE group a mean of 18 perivascular infiltration sites/ CNS was observed ,mean bradykinin level was 97 pg/ml.Control had no inflammation sites and a mean level of bradykinin of 21 pg/ml.In a second experiment on 3 EAE and 9 controls we observed a mean of 37 perivascular infiltration sites/ CNS in the EAE group and a mean bradykinin level of 216 pg/ml.The control group had no inflammation sites and a mean bradykinin level of 94 pg/ml.

Conclusion:Bradykinin blood level increase during EAE since bradykinin is a very active vasodilatating peptide it is possible that bradykinin contibute to the increase in blood-brain-barrier permeability observed in EAE.

242.12 INTERACTION OF COMPRESSION AND CONTACT VELOCITY IN DETERMINING THE SEVERITY OF SPINAL CORD INJURY. T.E. Anderson. Biomedical Science Department, General Motors Research Laboratories, Warren, MI 48090-9058.

Previous experimental models for spinal cord contusion injury did not allow independent control of compression and contact velocity. Interpretation of experimental data relating kinematics of vertebral injury to potential for spinal cord injury and loss of conduction requires a better understanding of the respective roles of compression and velocity in defining injury severity, both functionally and histologically. Our controlled dynamic compression model for experimental spinal cord injury was used in this study to evaluate the interaction of compression and contact velocity as independent variables, relative to injury severity.

Cord conduction was assessed using somatosensory evoked potentials in response to hindlimb stimulation. Latency to the first positive peak was determined at 20, 120 and 240 min. post-contusion. The increase in latency was found to correlate with the amount of compression delivered, with a significant difference between control and 50% compression, and between 25% and 50% compression. Although a small increase in latency was observed at 10 m/s compared to 0.6 m/s contact velocity, the difference was not statistically significant.

The extent of hemorrhagic necrosis was also assessed, and found to correlate with contact velocity. Such an injury is poorly modeled with weight-drop injury techniques, but can be addressed using the controlled contusion technique.

242.13 EARLY PATHOLOGICAL EFFECTS OF TRIMETHYLTIN (TMT) ON THE BASKET CELLS IN THE FASCIA DENTATA OF THE HIPPOCAMPAL FORMATION. L.W. Chang and R.S. Dyer*. Dept. of Pathology, Univ. of Ark. for Med. Sci., Little Rock, AR 72205 and Neurotoxicology Division, EPA Health Effects Lab., Research Triangle Park, NC 27711.

TMT is found to be a potent neurotoxicant with significant pathological impact on the limbic system, particularly the hippocampal formation. Electrophysiological investigations suggested that TMT may reduce recurrent inhibition of the basket cells in the dentate gyrus resulting in hyperactivation of the dentate granule cells (Dyer et al., Soc. Neurosci. Abstr. 8:82, 1982). Biochemical data also indicated that the inhibitory GABA system in the brain was also disrupted by TMT (Doctor et al., Toxicol. 25:213, 1982). Our present study was designed to examine the pathological changes in the basket cells (inhibitory neurons) in the dentate gyrus under the toxic influence of TMT. Male Long-Evans rats were injected (i.p.) with trimethyltin chloride (TMT-Cl) at the dosage of 6.0 mg TMT-Cl/kg b.w. Animals, in groups of four, were sacrificed at 24 and 72 hours after TMT exposure. All animals were perfused intracardially with saline solution followed by 2.5% buffered glutaraldehyde. The hippocampal formations were dissected out carefully and further fixed for light and electron microscopy examination. At 24 hours, no remarkable light microscopic changes were observed among the dentate granule cells, the basket cells, and the pyramidal neurons of the Ammon's horn. By 72 hours, scattered neuronal swelling and necrosis was observed in the CA_{3c} neurons. With electron microscopy, however, significant morphological changes were demonstrable among the dentate basket cells as early as 24 hours after intoxication. Severe vacuolation and breakdown of the Golgi complex and endoplasmic reticulum characterized the early changes. Edema of some basket cells and synaptic terminals were also evident. No remarkable degenerative changes were observed among the dentate granule cells and Ammon's horn neurons at this time. By 72 hours, most of the edematous changes in the basket cells had subsided, however, large accumulation of lysosomes and increased density of the rough endoplasmic reticulum were found in many basket cells. Some basket cells also showed signs of degeneration. This study provided the first evidence of early changes in the dentate basket cells as a result of TMT poisoning. Changes of these inhibitory neurons may alter the function of the limbic system leading to subsequent morphological and functional changes in this area.

REGULATION OF PITUITARY FUNCTION IV

243.1 HYPOPHYSIOTROPIC REGULATION OF ACTH SECRETION IN RESPONSE TO INSULIN-INDUCED HYPOGLYCEMIA. P.M. Plotsky, T.O. Bruhn* and S. Otto*. Peptide Biology Laboratory, The Salk (CRF), arginine vasopressin (AVP) and epinephrine (E) in mediation of ACTH secretion in response to hypoglycemia. Systemic administration of 0.1 IU (iv) insulin in urethane-anesthetized, overnight-fasted male rats resulted in a 70% decrease of peripheral plasma glucose from a mean concentration of 139 ± 18 mg/dl (n=6). This was associated with a maximal 2.5-fold elevation of mean systemic ACTH concentrations within 30 min of insulin injection. The ACTH-secretory response was dependent upon the presence of CRF, as treatment with antiserum rC-70 to synthetic rat CRF (0.5 ml, iv; n=6) abolished the response. Pretreatment with the AVP antagonist deaminopenicillamine, 2-(O-methyl)tyrosine AVP (150 ug/kg, ip; n=7) greatly attenuated the response, while ganglionic blockade with chlorisondamine (3 mg/kg, ip; n=7) resulted in only mild attenuation of ACTH secretion. The hypoglycemic response was unaffected by these treatments. These observations led us to the hypothesis that hypoglycemia-induced elevation of systemic ACTH concentration would be positively correlated with hypophysial portal plasma irCRF concentration. Portal blood was obtained in sequential 30 min collections and assayed for irCRF and irAVP in a test of our hypothesis. Hypoglycemia was not associated with any significant deviation of portal plasma irCRF levels from an initial mean concentration of 348 ± 33 pg/ml (n=5); however, portal plasma irAVP levels were elevated 1.8-fold above the initial mean concentration. In the current paradigm, we believe that ACTH secretion is conditional upon the presence of CRF, which acts in a permissive fashion to allow expression of the ACTH-releasing activity of AVP and, possibly, E. This is in contrast to the dynamic roles of CRF, AVP and E in mediation of hemorrhage-induced ACTH secretion.

243.2 CORTICOTROPIN RELEASING FACTOR RELEASE FROM THE MEDIAN EMINENCE IN VITRO: EFFECT OF ADRENALECTOMY. M.C. Holmes*, F.A. Antoni*#, K.J. Catt* and G. Aguilera*. (SPON: M.G. Miller) ERRB, NICHD and #LCB, NIMH, National Institutes of Health, Bethesda, MD 20205.

Corticotropin releasing factor (CRF) synthesized in the paraventricular nucleus is released from terminals in the median eminence (ME), into the portal blood. CRF is accumulated in the ME suggesting that secretion of the peptide could be regulated at this level. In an attempt to determine the mechanisms of control of CRF secretion, we developed a system to measure the release of CRF from the ME in vitro. CRF release in response to high concentrations of potassium (K+) in ME removed from control or 4-day adrenalectomized (ADX) rats was analyzed in an effort to determine if CRF release from isolated nerve terminal could account for increased ACTH levels following ADX. Median eminences were incubated in a Krebs-Ringer bicarbonate solution (KRBG) or KRBG containing high concentrations of K+. The medium was replaced every 15 min for a total incubation time of 6 hours. CRF secretion was measured in the medium by radioimmunoassay. Basal secretion was undetectable, and increasing concentrations of K+ (20-60 mM) elicited dose-dependent increases in CRF release. When calcium free-KRBG plus 2 mM EGTA was substituted for KRBG, no effect of K+ (60 mM) was observed, indicating that a calcium dependent secretory mechanism is involved. In ME removed from 4-day ADX rats, K+-stimulated CRF release was markedly decreased (2.47 ± 0.55 fmol/ME compared to 7.7 ± 1.62 fmol/ME in controls). Injection of 50 or 100 µg dexamethasone s.c./rat/day reversed the effect of ADX on K+-stimulated CRF release to give responses similar to those of control tissue. The content of CRF in the median eminence of ADX animals was reduced to 43% of controls (sham, 537 ± 61 fmol/ME; 4-day ADX, 232 ± 29 fmol/ME; mean CRF ± SEM; n=5), probably as a result of increased CRF turnover after ADX. Dexamethasone treatment returned the CRF content to control levels (594 ± 29 fmol/ME). The results show that K+ evoked CRF release from the ME in vitro is related to CRF content. Hence, it can be suggested that the enhancement of CRF release upon adrenalectomy is the result of increased activity of the CRF cell bodies and is not accompanied by a marked alteration of the function of nerve terminals in the ME.

243.3 PROSTAGLANDIN-DEPENDENT AND INDEPENDENT HYPOTHALAMIC-MEDIATED ACTH SECRETION IN VITRO. E. Redei*, B.J. Branch* and A.N. Taylor. Dept. of Anatomy and BRI Lab. of Neuroendocrinology, UCLA and West L.A. VA Med. Ctr., Brentwood Div., Los Angeles, CA 90024.

We are studying the mechanisms underlying the discharge of ACTH in response to stress in an in vitro system. Our previous data indicated that prostaglandins (PG) are involved in the negative feedback action of corticosterone (Cpd B) on the hypothalamus (H)-pituitary (P)-adrenal (A) axis. In this study we perfused H, anterior P, and A fragments and collected 2-min fractions from P and A, in which ACTH and B, respectively, were measured by RIA. Backflow (feedback) to H was established with 1/3 of the perfusate from A. To study the role of PG and its mode of action, three stages in the stimulus-secretion coupling of the HPA axis were investigated: Cpd B inhibition of the release of arachidonic acid (AA), a PG precursor, and its reversal by AA; PGE biosynthesis and its inhibition; and adrenergic receptor mediation of PG actions. Acetylcholine (Ach), added to H, was used as a stimulus for the HPA axis.

Ach produced four surges of ACTH: the first (peak 1) at 6-8 min, the second (peak 2) at 10-12 min, the third (peak 3) at 16-18 min and the fourth (peak 4) at 28 min after addition of Ach. PGE_2, added to H, produced peaks 1, 3 and 4. PG biosynthesis inhibitors (aspirin and indomethacin) blocked Ach-induced peaks 1, 3 and 4, without affecting peak 2. CRF added to P produced mainly peak 2. Cpd B, added to H, abolished Ach-induced peak 2, while partially inhibiting peaks 1, 3 and 4. Feedback from A inhibited all Ach-induced peaks to varying degrees. AA in the presence of Ach and feedback from A reversed the feedback effects on peaks 1 and 4 and partially on peak 3. PGE_2 and the β-adrenergic blocker, propranolol, inhibited peak 1 without affecting peaks 3 and 4, while the α-blocker, Prazosin, only inhibited peak 3.

These data support the existence of multiple mechanisms in the HPA response to stress. It appears that the first ACTH peak involves a β-adrenoceptive mechanism; that CRF produces the second peak; that the third peak represents an α-adrenoceptive mechanism; and that the fourth peak is probably directly mediated by PG. Further, it appears that depletion of AA by corticosteroids may be partially responsible for the negative feedback actions of Cpd B on the hypothalamus. (Supported by NIH-HD 07228 and VA Medical Research Service.)

243.4 INVOLVEMENT OF ARACHIDONIC ACID METABOLITES IN ACTH RELEASE. A. Luini* and J. Axelrod, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20205.

The mouse pituitary tumor cell line AtT-20 secretes ACTH in response to a variety of agents including corticotropin releasing factor (CRF), isoproterenol, dibutyryl cAMP, the Ca^{++} ionophore A23187 and potassium as well as melittin and phorbol myristate acetate. The latter two agents are known activators of phospholipase A_2, the enzyme that liberates arachidonic acid from membrane lipids. This suggested that arachidonic acid or some of its metabolites are required for ACTH secretion. The phospholipase A_2 blockers mepacrine and p-bromophenacyl bromide inhibited the ACTH release stimulated by CRF or isoproterenol with IC_{50}'s of 50 μM and 80 μM respectively. Metabolism of free arachidonic acid can occur through three enzymatic pathways, the cycloxygenase, the lipoxygenase and the epoxygenase. Indomethacine (1-100 μM), a cycloxygenase blocker, did not affect basal and CRF (or isoproterenol) stimulated ACTH release, whereas nordihydroguaieretic acid (NDGA) (IC_{50} = 12 μM) and butylated hydroxytoluene (IC_{50} = 22 μM), both lipoxygenase inhibitors, abolished the stimulation of ACTH release produced by the two secretagogues. Since CRF and isoproterenol enhance release via an increase in cAMP generation we tested whether this effect would be reduced by the above phospholipase and lipoxygenase inhibitors. None of these compounds changed the basal or stimulated cAMP levels with the exception of mepacrine (200 μM). NDGA (100 μM) also abolished the release induced by dibutyryl cAMP and potassium and reduced the stimulation by phorbol myristate acetate, melittin and compound A23187. These compounds do not increase cellular cAMP levels. The results suggest that lipoxygenase products are involved in ACTH release at a step distal to cAMP generation. From preliminary experiments on arachidonic acid metabolism using HPLC chromatography, it appears that lipoxygenase and epoxygenase products are quantitatively prominent in unstimulated cells and that exposure of the cells to 10^{-7}M CRF elevates the levels of several as yet unidentified metabolites.

243.5 THE ROLE OF HYPOPHYSIAL PORTAL PLASMA CORTICOTROPIN-RELEASING FACTOR, VASOPRESSIN, AND OXYTOCIN IN THE HYPOTHERMIA-INDUCED INHIBITION OF CORTICOTROPIN RELEASE. D.M. Gibbs, Dept. of Repro. Med., UCSD, La Jolla, CA 92093.

Although corticotropin (ACTH) secretion is usually stimulated by cold exposure in conscious animals, true hypothermia in hibernating or anesthetized animals is associated with inhibition of ACTH release. This was confirmed in pentobarbital-anesthetized male rats placed on ice. ACTH levels decreased significantly within 15 minutes in parallel with a decrease in body temperature. To investigate the mechanism by which ACTH secretion is inhibited, hypophysial portal blood was collected from euthermic and hypothermic rats and the concentrations of CRF, vasopressin (AVP) and oxytocin (OT) were measured by RIA. Both AVP and OT can modulate the secretion of ACTH by enhancing the pituitary responsiveness to CRF (Vale et al., Endocrinology 113:1121, 1983). Whereas CRF levels in portal plasma were not different in the two groups (euthermic: 447±104 pg/ml; hypothermic: 570±66 pg/ml), AVP (euthermic: 1.28±.21 ng/ml; hypothermic: 0.84±0.08 ng/ml) and OT (euthermic: 1.38±0.18 ng/ml; hypothermic: 0.94±0.09 ng/ml) levels were significantly lower in hypothermic rats ($P<0.05$). The concentration of AVP and OT in peripheral plasma was also significantly lower in hypothermic rats compared to euthermic controls.

The pituitary responsiveness to CRF during hypothermia was tested in vivo and in vitro. In pentobarbital-anesthetized male rats injected iv with 0.1 or 1.0 nmoles of CRF, the ACTH response was significantly smaller in hypothermic compared to euthermic animals. However, hemipituitaries superfused at 31°C released the same amount of ACTH in response to 1 nM CRF as hemipituitaries superfused at 37°C (31°C: 541±90 pg; 37°C: 563±29 pg) despite reduced baseline secretion (31°C: 77±10 pg/10 min; 37°C: 114±14 pg/10 min; $p<0.05$).

The data suggest that the inhibition of ACTH secretion during hypothermia is mediated by decreased hypothalamic secretion of AVP and OT which in turn decreases the pituitary responsiveness to CRF.

This work was supported in part by NIH grant AM32517 and a Mellon Foundation Faculty Scholar Award.

243.6 COLOCALIZATION OF CCK AND CRF IN THE HYPOTHALAMIC NEUROSECRETORY SYSTEM: A POSSIBLE ROLE IN THE REGULATION OF ACTH RELEASE E. Mezey, T.D. Reisine, L.R. Skirboll, M. Beinfeld, J.Z.Kiss. Lab. of Cell Biol. and Clin. Neuroscience Branch, NIMH, Bethesda, MD 20205 and Dept. Pharmacology, St. Louis Univ., St. Louis, MO 63104.

Cholecystokinin (CCK) has been demonstrated in magnocellular neurosecretory cells of the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus (Vanderhaegen, J.J. et al., Cell and Tiss. Res., 221:277, 1981). Recently, we reported that CCK is also present in parvocellular neurons of the PVN in overlapping distribution with CRF containing cells (Kiss, J. et al., J. Comp. Neurol., 1984).

Using indirect immunofluorescence histochemistry with the elution-restaining technique of Tramu et al., J. Histochem. Cytochem. 6:322, 1978, we obtained evidence that neurons in the parvocellular subdivision of the PVN contain both CCK and corticotropin releasing factor (CRF). Since these cells have been shown to provide innervation to the external zone of the median eminence (ME) where the portal cappilaries are located, we examined the roles of CCK, CRF and their combination on ACTH release from monolayer cultures of the anterior pituitary. CRF exerted a potent (EC50=1nM) and a pronounced stimulation of ACTH release from these cells. CCK stimulated ACTH release at concentrations of 10-100 nM but this effect was much less than that observed for CRF. When added simultaneously, CCK did not potentiate nor add to the ACTH release induced by 100nM of CRF. These data suggest that under certain physiologic conditions, CCK may act as a corticotropin releasing factor.

Finally, since neuroendocrine manipulation such as adrenalectomy (ADX) has been shown to effect ACTH and/or CRF synthesis/release, the effect of ADX on hypothalamic CCK levels were examined using RIA techniques. We found that ADX significantly reduced CCK levels in the PVN and ME while having no effect on the SON or posterior pituitary.

In summary, evidence is presented that CCK and CRF coexist in parvocellular neurons of the PVN. In addition, CCK has been shown to stimulate ACTH from pituitary cultures. Finally, ADX is not only effective in altering ACTH and CRF levels but also served to significantly reduce hypothalamic CCK levels. Thus, in addition to the magnocellular hypothalmic neurohypophyseal CCK system, there is a parvocellular CCK system which may be involved in regulating the release of ACTH.

243.7 **EFFECTS OF NEONATAL ADMINISTRATION OF MONOSODIUM L-GLUTAMATE (MSG) ON GONADOTROPIN SECRETION, GONADOTROPES AND MAMMOTROPES IN PREPUBERTAL FEMALE FATS.** M.O. Dada* and C.A. Blake (SPON: A.M. Earle). Department of Anatomy, University of Nebraska Medical Center, Omaha, NE 68105.

Administration of MSG to neonatal rats destroys perikarya in the hypothalamic arcuate nuclei, including most of the Group A12 dopaminergic cells and causes reproductive disorders. We have investigated the effects of MSG treatment to neonates on gonadotropin secretion and luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin (PRL) cells during the late prepubertal period in female rats. Rats were injected with MSG (4mg/g body weight) or saline on days 1,3,5,7 and 9 of life and were decapitated on day 35. Trunk blood was collected for radioimmunoassay of serum LH and FSH concentrations. Anterior pituitary glands (APGs) were bisected. One half was homogenized and assayed for LH and FSH. The other half was placed in culture medium to study the basal LH and FSH release rates. Pituitary sections from additional rats were immunostained for LH, FSH or PRL. APG weights and APG weights/body weights were reduced in the MSG-treated group. Yet, MSG treatment did not lower the serum LH or FSH levels, the APG concentration or content of LH or FSH, or the basal release rates of LH or FSH per mg APG or per entire gland. MSG treatment did reduce the cross-sectional areas of LH and FSH cells but the volume density, the numerical density and the percentage of APG cells that contained LH or FSH were unaffected by MSG-treatment. In both groups, all FSH cells also contained LH but there was a small percentage of gonadotropes that contained only LH. Surprisingly, the volume density of PRL cells was not altered in the MSG-treated group. The results suggest that in 35-day-old female rats treated neonatally with MSG 1) the percent of gonadotropes that are LH or LH/FSH cells are normal, 2) the LH and LH/FSH cells are smaller but contain the same amount of hormone(s) as those of LH or LH/FSH cells in normal rats, and 3) the gonadotropes are releasing sufficient hormone to maintain normal tonic levels of serum LH and FSH. We also found no evidence to suggest that the Group A12 dopaminergic cells play any role in controlling the volume density of prolactin cells in the APG.

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243.8 EFFECTS OF DAYLENGTH ON NUCLEAR ANDROGEN RECEPTOR OCCUPANCY IN NEUROENDOCRINE TISSUES OF THE GOLDEN HAMSTER. E. Bittman and L.C. Krey* Rockefeller University, New York, N.Y. 10021.

Daylength modulates the neuroendocrine effects of gonadal steroids in seasonal breeders. In male hamsters, low doses of testosterone (T) suppress serum LH levels in short days but not in long days. In contrast, higher levels of T are needed to activate male sexual behavior in short than long photoperiods. We determined whether photoperiod alters levels of translocated androgen+receptor complexes in nuclear pellets from preoptic area (POA), medial basal hypothalamus (MBH), pituitary (PIT) and seminal vesicle (SV) by exchange assay (Brain Res. 275:75). Groups of 36 hamsters were castrated in long days (14L:10D). Three weeks later, hamsters received no implants or were given Silastic T capsules calculated to maintain serum androgens within the low (5mm) or intermediate (7.5mm) physiological range. Half the hamsters were then moved to short days (10L:14D). After 56 days, we measured exchangeable nuclear androgen in tissues from pools of 3 hamsters. One group (SAT) was given additional T capsules to generate superphysiological T levels 48h before sacrifice. Serum LH and T levels were assayed in trunk blood.

Exposure of intact hamsters to short days reduced relative testis weight (5.7±1.0 mg/g b.w. in 10L:14D vs. 24.8±0.7 in 14L:10D, mean±SEM, $p<.001$) and serum T. Exchangeable nuclear androgen receptor levels (fmole 3H-R1881/mg DNA) was correspondingly reduced in MBH (91±27 vs. 204±60, $.2>p>.1$), POA (80±26 vs. 216±22, $p<.01$), PIT (137±43 vs. 380±40, $p<.01$), and SV (376±324 vs. 1026±215, $p<.05$). Castrates receiving no T had little or no measurable nuclear androgen in any tissue studied. Serum LH levels were lower in T-treated castrates kept in short days. The 5mm, 7.5mm, and SAT capsules generally produced progressively increasing levels of exchangeable androgen in both short days (MBH 121±102, 254±35, and 653±238; POA 83±45, 249±12, and 415±21; PIT 161±26, 295±23, and 405±49; SV 384±68, 832±38, and 916±77, respectively) and long days (MBH 105±27, 244±13. and 516±115; POA 110±32, 251±31, and 655±77; PIT 252±26, 483±33, and 511±19; SV 404±66, 1036±165, and 1023±40, respectively). Daylength did not affect MBH or SV androgen receptor occupancy at any dose of T; levels were elevated in long day PIT of 5mm and 7.5mm groups and in POA of the SAT group ($p<.05$). These results do not support the hypothesis that short days magnify T negative feedback by increasing translocation in neuroendocrine tissues. Such a mechanism may, however, contribute to photoperiodic modulation of behavioral effects of T. Supported by NIH grant NS20279.

243.9 LOSS OF SEX DIFFERENCE IN GONADOTROPIN RELEASE IN RATS TREATED WITH AN OPIATE ANTAGONIST DURING EARLY LIFE. D.K. Sarkar,* and S. Lira* (SPON: W.A. Harris). Dept. of Repro. Med., Univ. of California, San Diego, La Jolla, CA 92093.

The role of opiates in the sexual differentiation of the brain was tested in rats by injecting the opiate antagonist naltroxone during the critical period of sex differentiation of the brain and studying the release of gonadotropin during pubertal period. Newborn Sprague-Dawley rats were given daily subcutaneous injection of naltroxone (50 mg/kg) in saline or saline (0.1 ml) until 7 days of age. After weaning on day 21, some of the animals were injected with estradiol benzoate (EB; 1 µg/100 g B.W.) or oil (0.1 ml) on day 21 and day 23 after birth. Following day, these rats were sacrificed between 1600-1630h, plasma and pituitary LH levels and hypothalamic and preoptic LHRH concentrations were determined. The rest of the animals were kept in the animal house and inspected daily for vaginal opening until 45 days after birth.

The concentration of plasma LH was significantly increased after EB treatment in female rats but not in male rats. However, administration of naltroxone during the critical period completely abolished EB-induced LH surge in 60% of the females and partially reduced EB-induced LH release in the rest of the female rats. Pituitary LH concentration in female rats were slightly higher than in males. Naltroxone did not change the concentration of pituitary LH in female and male rats. The concentration of LHRH in the preoptic area was lower in both sexes of rats after naltroxone treatment when compared with that in saline-treated groups. Hypothalamic LHRH levels were similar in both males and females treated with naltroxone or saline. Ovariectomy on the day of the birth did not prevent the inhibitory action of naltroxone on EB-induced LH release nor block the reduction of LHRH in the preoptic area by the antagonist. Naltroxone-treated animals also showed no sign of vaginal opening (puberty) until at least day 47, whereas saline-treated animals had vaginal opening between days 36-37 after birth. These results suggest that (1) opiate antagonist administration during the critical period can prevent the differentiation of female cyclic brain possibly by a direct action on the brain, (2) endogenous opiates may be involved in the sexual differentiation of the brain.

(Supported by Mellon Foundation Faculty Scholar Award to D.K.S.)

243.10 EVIDENCE OF TACHYPHYLAXIS-LIKE RESPONSES OF DISPERSED PITUITARY CELLS TO ZEARALENONE AND ESTRADIOL. G.E. RESCH* (SPON: A. DICK). Dept. of Biol. & Schl. of Med., Univ. of Missuori - Kansas City, Kansas City, MO 64110.

Efforts to characterize a previously reported zearalenone (F2) stimulation of prolactin release from dispersed pituitary cells, suggest a tachyphylaxis-like response. Equimolar doses of F2 and estradiol were administered to a continuously perfused column of trypsin-dispersed rat pituitary cells. Perfusion media were made using tissue culture medium 199 with glucose and agonists as appropriate. Media at 37°C were bubbled with O_2:CO_2 (95:5) to maintain the pH at 6.8 to 7.0. Agonists were injected onto the column serially at time intervals sufficient to allow each prolactin response to return to baseline. Response curves were corrected for latency between injection site and the column cell layer.

The data indicate successive responses serially administered agonists differed by 1) increased latencies, 2) decreased amplitudes and 3) increased durations. Prolactin responses to equimolar doses of estradiol were qualitatively similar. The results indicate that comparisons of responses elicited in this experimental preparation should be interpreted with caution. (Supported in part by Weldon Spring Grant No. K3-40110).

243.11 PINEALECTOMY DURING INFANTILE DEVELOPMENT FAILS TO INTERRUPT THE PREPUBERTAL HIATUS IN GONADOTROPIN SECRETION IN THE MALE RHESUS MONKEY (MACACA MULATTA). T.M. Plant and D.S. Zorub*. Depts. Physiology & Neurological Surgery, Univ. of Pittsburgh Sch. of Med. and Div. Neurological Surgery, Shadyside Hospital, Pittsburgh, PA 15261.

In higher primates, the postnatal ontogeny of gonadotropin secretion is characterized by elevations in plasma concentrations of LH and FSH during infantile and postpubertal development that are separated by a prolonged phase of diminished gonadotropin release during much of prepubertal development. The prepubertal hiatus in gonadotropic activity, which underlies the protracted delay in the onset of puberty in higher primates, appears to be occasioned by a non-gonadal restraint of hypothalamic GnRH secretion. In order to determine the role, if any, of the pineal gland in initiating the arrest of the hypothalamic-gonadotroph axis, 6 neonatally orchidectomized rhesus monkeys were pinealectomized at 4-6 weeks of age using microneurosurgical tecnnique. The patterns of LH and FSH secretion during infantile development (birth until 35 weeks of age) were monitored in the pinealectomized animals by RIA of plasma samples collected at weekly intervals, and then compared to those in 2 similar animals subjected to a sham procedure and to those previously established for neonatally orchidectomized animals with intact central nervous systems (Endocrinology 106:1451, 1981). The pinealectomized animals were subsequently sacrificed and the completeness of the procedure established by histological evaluation. The pattern of gonadotropin secretion during infantile development in the pinealectomized animals did not differ from that in sham operated subjects, and in both groups the time courses of LH and FSH secretion were similar to those previously described for agonadal neonates with intact nervous systems. Most notably, the elevated open-loop levels of LH and FSH that are characteristically observed during early neonatal life in this species began to decline at 2 months of age in the pinealectomized animals, as in the sham and control groups, reaching by 34 weeks of age concentrations that were no longer detectable by RIA. The failure of pinealectomy to abolish the prepubertal hiatus in open-loop LH and FSH secretion, and presumably therefore to interupt the restraint of hypothalamic GnRH release, provides compelling evidence against the view that the pineal gland is a major determinant of the timing of the onset of puberty in higher primates. Supported by NIH Grants HD13254 and 08610.

PINEAL GLAND

244.1 PERSISTENCE OF PHOTIC RESPONSES IN THE PINEAL GLAND AFTER ITS PEDUNCULOTOMY AND GANGLIONECTOMY OF THE CERVICAL SYMPATHETIC. C. Barajas-López*, M.A. Barrientos-Martínez*, M.V. García-Garduño* and C. Reyes-Vázquez. Depto. de Fisiología, Div. de Investigación, Fac. de Medicina, UNAM. México 04510, D.F., MEXICO.

Electrophysiological studies have shown that the last components of the visual evoked potentials (VEPs) recorded in the pineal gland (PG) disappear after bilateral extirpation of the cervical sympathetic ganglia (CSG). However, in another similar study no modifications were found after the same maneuver, whereas the transection of the pineal stalk abolished the VEPs completely. In the present paper, we analize, simultaneously, the participation of the pineal stalk and of CSG in the generation of VEPs in PG. We recorded VEPs in PG, lateral hipothalamus (LH), and habenular complex (HC), the latter two structures are supposed to be involved in the transmission of visual information to PG. Semi-microelectrodes were implanted in male anesthetized rats in the aforementioned structures by conventional stereotaxic techniques. In some rats a lesioning electrode was implanted in the pineal stalk and in others it was transected. The VEPs were recorded using a commuter, which allowed free movement. The potentials were amplified, recorded, and averaged by conventional electrophysiological techniques. Visual stimulation were flashes delivered by a Grass photostimulator (PS22). Recordings were begun 2 to 3 days after the implantation. Three recording sessions were performed (one every 24 hrs) before and after extirpation of CSG or electrolytic lesion of pineal stalk. The VEPs recorded in LH and HC were characterized by 3 components (PNP). The latencies of the first waves were of 33 and 34 ms, respectively, whereas the recordings in PG had a different polarity (NPN) and the first component had a latency of 36 ms. The VEPs persisted in all three structures after the extirpation of CSG or electrolytic lesion of the pineal stalk. VEPs were also observed in the pineal stalk transected rats. Electrode placements were determined after the last recordings by histological sections.

These results suggest that the VEPs recorded in PG are not generated in this structure, since they persist after interrupting its connections to the rest of the central nervous system.

244.2 HAMSTER AND RAT PINEAL GLAND β-ADRENOCEPTOR CHARACTERIZATION WITH IODOCYANOPINDOLOL AND THE EFFECT OF DECREASED CATECHOLAMINE SYNTHESIS ON THE RECEPTOR. C.M. Craft, W.W. Morgan, [1]D.J. Jones, and R.J. Reiter, Department of Cellular and Structural Biology and [1]Department of Anesthesiology, The Univ. of TX Hlth. Sci. Ctr. at San Antonio, San Antonio, TX 78284.

Although catecholamine content does not vary during the 24 hr period in the hamster pineal, in both the rat and hamster, catecholamine synthesis is greater in the dark (Neuroendocrinology 38:193-194, 1984). Further studies of hamsters exposed to continuous light (LL) for 36 hrs, after superior cervical ganglia decentralization or ganglionectomy (SCGX) produced a significant decrease in pineal catecholamine synthesis and therefore decreased noradrenergic neuron activity in the dark compared to controls (C) ($p<0.001$). The depletion of norepinephrine was never complete in any experimental group. Even though these experimental conditions decrease noradrenergic neuron activity, they do not induce postsynaptic supersensitivity, determined by increases in melatonin content, in the hamster pineal when exogenous norepinephrine is administered. This is in direct contrast to the results obtained in experiments with rats. Therefore, pineal β-receptors in hamster and rat were studied to determine possible mediation of this difference. The new β-specific radioligand, iodocyanopindolol, was utilized to characterize the pineal β-adrenergic receptor in both species. Under equilibrium conditions, the receptor was saturable, and the ligand demonstrated selectivity and stereospecificity in both species. The density of receptors in the rat pineal was 15-fold greater than that in the hamster pineal while the affinity of the rat pineal receptor was one-third that of the hamster. Utilizing this receptor binding technique, it was determined that decreased catecholamine synthesis did not alter receptor density or affinity of β-receptors in the hamster pineal (Bmax: C, 55±6 vs SCGX, 45±14 vs LL, 53±4 fm/mg protein; KD: 0.052±0.003 nM. In contrast, the density of the β-receptor increased in the rat pineal with no change in affinity. (Bmax: C, 596±17 vs SCGX, 816±20 fm/mg protein; C, 548±13 vs LL, 621±9 fm/mg protein ($p<0.05$); KD: 0.15±0.02 nM. This provides a plausible explanation for the variability of postsynaptic responses in the two species. (Supported by NSF grant #PCM 8304706 to RJR, NIH grant #DA 00755 & #DA 00083 to WWM & NIH-NINCDS grant #14546 to DJJ.)

244.3 AMINERGIC EFFECTS ON RAT PINEAL UNIT ACTIVITY, C. Reyes-Vazquez, B. Prieto-Gomez* and N. Dafny (SPON: R. Wiggins), Depto. Fisiologia, Fac. Med. UNAM, Mexico 04510, and Neurobiology Department, University of Texas Medical School at Houston, Houston, Texas 77025.

Most pineal studies have used pharmacological, chemical or surgical interruption of the pineal sympathetic neural input, to study its properties. There are no neurophysiological studies investigating the electrophysiological properties of the rat pineal related both to superior cervical ganglion (SCG) input and norepinephrine (NE) application. The present study was initiated to determine the electrophysiological relationship between the effects of SCG stimulation and local application of NE on pineal electrical discharges. Seventy six pineal units were recorded from 26 anesthetized rats. About 50% of the units showed an average spontaneous firing rate lower than 14 spikes/sec. SCG stimulation caused excitation in 42/76 of the recorded pineal units. Six of these units did not exhibit spontaneous activity. Microiontophoretic application of NE affected 61% of the pineal units tested. Two patterns of activity following NE ejection were observed: increases of firing rate (64%) and decreased in firing rate (36%). NE-induced excitation was observed only in those units excitated by SCG stimulation. When NE and SCG stimulation were applied together, partial summation of the excitation induced by each one alone was observed. All of the units affected by NE with a decrease in firing rate failed to respond to SCG stimulation. In most cases, the local application of propranolol (NE-antagonist) significantly blocked both, the excitation initiated by SCG stimulation (69%) as well as the activation induced by NE (81%). Moreover, propranolol also was able to block the NE induced decreases of pineal unit activity.

244.4 **PINEAL MELATONIN SYNTHESIS IN THE HAMSTER: SENSITIVITY TO LIGHT.** **D. J. Hudson and M. Menaker. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.**

We have previously identified novel photoreceptor properties of the circadian phase shifting system of the golden hamster (Takahashi et al.,1984,Nature 308:186). The spectral sensitivity of the phase shifting response has a maximum near 500 nm, and is very similar to the absorption spectrum of rhodopsin. However, the threshold of this response is quite high, and the reciprocal relationship between intensity and duration holds for very long durations. The photoreceptive system that mediates phase shifting appears to be markedly different from that involved in visual image formation. To investigate the relationship between circadian and photoperiodic (reproductive) photoreception we examined the effects of light on the hamster pineal gland.

We measured the acute effects of light pulses of different intensities (covering approximately 3 log units) in suppressing pineal melatonin. Male Syrian hamsters held in long days (LD 14:10) were exposed to 4 minute light pulses (515 nm) during the night (at CT 18, a time at which we have also measured an intensity-response curve for phase shifting), returned to the dark for a period of 30 minutes, and sacrificed. Pineals were removed and frozen for subsequent melatonin extraction and measurement by radioimmunoassay. A dark control group was handled and placed into the light pulse apparatus but was not exposed to light. A light control group was exposed to bright fluorescent light for the entire 30 minutes before sacrifice.

Pineal melatonin suppression is not an "all-or-none" response to light exposure, but is graded with light intensity. The sensitivity of this pineal response is at least 2 log units greater than the sensitivity of the circadian phase shifting system. Perhaps there exist pathways by which light affects pineal melatonin synthesis directly without shifting the circadian clock. If so, it might be possible to dissociate the effects of light on reproductive state from its effects on the circadian system by the use of light pulses of carefully chosen intensity. In preliminary experiments we have delayed testicular regression (after transfer of animals from long days into darkness) by the repeated presentation of light pulses which do not cause phase shifts in the locomotor rhythm.

Supported by NIH HD13162.

244.5 MATURATION OF MELATONIN RHYTHMS IN FEMALE LAMBS UNDER ARTIFICIAL AND NATURAL PHOTOPERIODS. S.M. Yellon* and D.L. Foster. Reproductive Endocrinology Program, The University of Michigan, Ann Arbor, MI 48109.

The ability to recognize environmental photoperiod is proposed to depend upon development of a stable, photoperiod-entrained pineal melatonin rhythm. In the female sheep, this rhythm provides important information about daylength which is used to time puberty (ca., 25-35 weeks). The present report describes certain features of the serum melatonin pattern during sexual maturation. March-born lambs were reared under continuous artificial photoperiods, ie., long days (15L:9D, n=5) or short days (9L:15D, n=5), or outdoors under changing natural daylengths (n=6). Blood samples (n=12) were collected at 1-3 h intervals over a 24-h period every 4 to 8 weeks beginning at 3 or 6 weeks of age. Serum melatonin concentrations were determined by radioimmunoassay. A typical 24-h melatonin rhythm in postpubertal lambs (n=10) under natural short days was characterized by low daytime levels (32 ± 2 pg/ml, mean ± SE, 5 samples) and a nighttime rise (362 ± 50 pg/ml, 7 samples) that was proportional to the duration of darkness. In lambs under artificial photoperiods, day/night differences in serum melatonin were not observed at 3 (short days) or 6 (long days) weeks of age. Differences were evident by 10 weeks of age under both long days (day, 30 ± 4 pg/ml, 7 samples; night, 142 ± 42 pg/ml, 5 samples) and short days (day, 39 ± 5 pg/ml, 5 samples; night, 194 ± 28 pg/ml, 7 samples). By contrast, in lambs under natural photoperiods, a distinct day/night melatonin rhythm was not observed at 6, 10 or 16 weeks of age; for example at 10 weeks of age: day= 138 ± 43 pg/ml (7 samples); night= 162 ± 36 pg/ml (5 samples). By 22 weeks of age, melatonin rhythms reflecting the duration of darkness, were established (day, 24 ± 5 pg/ml, 6 samples; night, 144 ± 20 pg/ml, 6 samples). At ages when day/night differences were not established, < 10 weeks under artificial photoperiods and < 22 weeks in natural photoperiods, melatonin patterns were characterized by inconsistently high serum levels during both day and night. These results indicate that day/night differences in melatonin secretion develop earlier in lambs raised under artificial photoperiods compared to natural photoperiod. It remains to be determined which properties of artificial photoperiod are responsible for the advanced onset and stabilization of the melatonin rhythm. (Supported by NIH HD-06471, HD-11311 and HD-18394.)

244.6 HORIZONTAL KNIFE CUTS IN THE SUPRACHIASMATIC AREA PREVENT GONADAL RESPONSES TO PHOTOPERIOD. G.A. Eskes* and B. Rusak (SPON: I.A. Meinertzhagen). Dept. of Psychology, Dalhousie Univ., Halifax, Nova Scotia.

Pineal regulation of photoperiodic effects on gonadal function is mediated by a retinal projection that terminates in the suprachiasmatic nuclei (SCN). The SCN appear to project to the pineal gland via a dorsal periventricular route that terminates in the paraventricular nucleus (PVN). Lesions dorsal to the SCN (Eskes, G.A. et al., Biol. Reprod., in press, 1984) or PVN lesions (Klein, D.C. et al., Brain Res. Bull. 10: 647, 1983; Pickard, G.E. & Turek, F.W., Neurosci. Lett. 43: 67, 1983) prevent pineal responses to photic input and gonadal regression during short day exposure. Interpretation of the effects of lesions dorsal to the SCN is difficult since these lesions disrupt periventricular cell groups as well as SCN efferents. We studied the role of the SCN dorsal projection to the PVN in hamster photoperiodism by interrupting this pathway by horizontal knife cuts which presumably leave most cell groups intact.

Hamsters with sham operations (Shams), horizontal knife cuts aimed at the periventricular area (PVA) dorsal to the SCN (Cuts) or lesions of the PVN (PVNx) were held in short days (LD10:14) for 91 days. All males were laparotomized on days 60 and 91 for testis measurement. Testis lengths (TL) of Cut animals were greater ($p < .05$) than those of Shams on days 60 (TL=15.6 ± .9mm vs 12.5 ± .7mm, $\bar{X}$ ± SEM) and 91 (TL= 15.4 ± .9mm vs 12.0 ± .5mm). Testes of 8/13 animals with cuts exhibited incomplete or no regression after 91 days (TL=17.8 ± .5mm). Horizontal cuts effective in blocking regression were found in 2 separate areas: 1) dorsal to and extending over the entire rostrocaudal extent of the SCN, and 2) in the ventral preoptic area extending caudally only to the anterior border of the SCN. PVN lesions also affected gonadal responses (TL=16.1 ± 1.1mm on day 60 and 14.9 ± 1.1mm on day 91, $p < .05$). The gonads of 8/13 males with damage to the PVA and medial portions of the PVN exhibited no or incomplete regression after 91 days (TL=18.6 ± .7mm).

These data confirm the involvement of the PVN in hamster gonadal responses to photoperiod and suggest the existence of 2 pathways near the SCN which are critical for photoperiodic responses; one extending from the SCN to the PVN and a second in the POA. POA cuts may have interrupted the identified reciprocal connections of the MPOA and SCN, or they may have affected structures independent of the SCN.

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244.7 ULTRASTRUCTURAL CORRELATES TO THE SUMMER RISE IN PINEAL ARGININE VASOTOCIN (AVT) ACTIVITY: A MORPHOMETRIC ANALYSIS OF PINEALOCYTES IN MALE RATS. J.A. McNulty*, M.M. Prechel*, T.K. Audhya*, D. Taylor*, L. Fox*, T.A. Dombrowski* and W.H. Simmons*. (SPON: F. LaVelle). Departments of Anatomy and Biochemistry, Loyola Univ. Med. Ctr., Maywood, IL 60153.

A significant and predictable rise in AVT-immunoreactivity has been reported in the pineal gland of rats during August (Prechel et al., Neurosci. Abstr. 730, 1983). The hypothesis that the pineal is the source of this AVT prompted the present study to determine if specific ultrastructural changes occur during this period that may be related to peptide synthesis and/or storage.

Sprague-Dawley male rats (King Animal Labs, Oregon, WI) were housed in environmental chambers illuminated by fluorescent lamps, and entrained for two weeks to a 12:12 L:D cycle. Food and water were available ad libitum. Ten animals, 36-43 days of age, were sacrificed at mid-light and mid-dark on 21 Jul, 6 Aug, 12 Aug, 18 Aug, and 8 Sep 1983. Pineal glands were removed and used for measurement of AVT by RIA (n=5)(Fernstrom et al., Endocrinol. 106:243, 1980) or for routine TEM (n=5). Micrographs of 6 grid spaces (300-mesh) were recorded for each specimen. Measurements of volume densities and number of nuclei, lipid inclusions, and RER stacks were made using a 100 point grid. Nucleolar size was estimated on an image analyzer. Number of dense vesicles (DCV) and synaptic ribbons (SR) in 3 grid spaces were also counted. Data were analysed by the Student's t-test.

Mean AVT-activity was at the lower limits of detection (<50 pg/gland) on 21 July, but increased by 6 Aug (234-479 pg/gland). The highest level of AVT-activity was recorded on 12 Aug (1,015-,1,062 pg/gland); then the level gradually declined during the latter part of Aug (754-907 pg/gland) and early Sep (147-164 pg/gland). Ultrastructural changes that were correlated with the AVT-activity included trends for a greater amount of RER stacks and lipid during daytime samples with peak values of these variables occuring on 12 Aug. The lipid was frequently related to the RER stacks. One specimen from 12 Aug had an unusually large number of "annulate lamellae". Nucleolar size and number of DCV were greatest on either side of the 12 Aug peak in AVT-activity. No trends were detectable with regard to SR and nuclei.

A functional relationship between pineal AVT-activity and structures involved in peptide/protein synthesis is suggested by these results. Lipid stores and the enigmatic "annulate lamellae" may also participate in these processes. Supported by NIH Grants AM30970, HL28710 and NS20252 to WHS.

244.8 INCREASES IN PINEAL SEROTONIN N-ACETYLTRANSFERASE AND MELATONIN CONTENT BY ACUTE INSULIN STRESS. T.H. Champney, D.S. Christie* and R. J. Reiter. Dept. Cell. and Struct. Biol., Univ. of Texas Health Sci. Ctr., San Antonio, TX 78284.

The principle neurotransmitter involved in serotonin N-acetyltransferase (NAT) and melatonin (MEL) regulation in rats is norepinephrine (NE), which increases in the plasma during stress. Stress related increases in pineal NAT and MEL content have been seen in rats that were immobilized or given a carbohydrate load, but not during swim stress. NE or its agonists do not stimulate pineal NAT or MEL production in Syrian hamsters. Therefore, it was of interest to compare the effects of acute insulin stress on pineal NAT and MEL content in male rats and hamsters. Both rats and hamsters were injected with either 5 IU/kg or 10 IU/kg of insulin (bovine), i.p. Eight animals of each dosage were killed at 1, 2 or 3 hrs after injection. Eight animals were killed at the onset of the experiment to serve as pretreatment controls. The pineal, left adrenal and trunk blood were collected. Adrenal and plasma catecholamines were determined by high performance liquid chromatography. The pineals were assayed for NAT activity (radioenzyme assay) and melatonin content (radioimmunoassay). The plasma glucose levels were also measured and were lower at 1, 2 and 3 hrs after both dosages of insulin in the rats. In the hamsters, plasma glucose levels were only depressed at 1 hr after the 10 IU/kg injection of insulin. Plasma NE was increased at 2 (hamsters) and 3 hrs (hamsters and rats) after both dosages of insulin. Plasma epinephrine (EPI) was increased at 1 (rats), 2 (rats), and 3 hrs (rats and hamsters) after both dosages of insulin. Plasma dopamine (DA) was unchanged in both species. Hamster adrenal catecholamine content remained unchanged throughout the experiment. Rat adrenal DA was increased and adrenal EPI was decreased at 2 and 3 hrs after both insulin dosages. Pineal NAT was unchanged and MEL content was depressed in hamsters, even though their plasma catecholamines were elevated. In rats, pineal NAT and MEL content were increased 3 hrs after insulin injection, the same time that increases in plasma NE were seen. These studies show that plasma catecholamine elevations accompany increased pineal NAT and MEL content in the rat and decreased MEL content in the hamster. Further studies, including the use of adrenalectomy to block these effects are being formulated. (Supported by NSF Grant No. PCM 8304706.)

244.9 TIME COURSE OF INTRAVENOUSLY ADMINISTERED 3H-MELATONIN IN MOUSE SCIATIC NERVE AND SPINAL CORD. S.S. Erlich, L.P. Weiner, M.H. Weiss* and P.J. Syapin. Depts. of Neurological Surgery and Neurology, University of Southern California School of Medicine, Los Angeles, CA 90033

Although a few previous uptake, bioassay, and axonal transport studies suggest that peripheral nerve may be a site of action for melatonin, this site, as well as the spinal cord, has remained little-explored. The mouse,too, has received little attention in uptake and localization studies, except for one report analyzing the half-life of intravenously injected 3H-melatonin in whole mouse. Therefore, the purpose of the present study was to analyze 3H-melatonin distribution in the mouse, and to compare the sciatic nerve and spinal cord to other anatomic sites.

Eighty 4 to 6 month old male C57BL/6J mice, housed under LD14:10 (on at 0500, off at 1900), received intravenous injections of a 3H-melatonin solution containing $4x10^6$ dpm. Injections were spread throughout the photophase, from 1018-1842. Mice were decapitated at 8 time points from 30 sec to 3 hr post-injection. Numerous nervous system and peripheral sites were processed for liquid scintillation counting. The % counts due to 3H-melatonin was determined by extraction procedures and thin-layer chromatography.

In whole blood, dpm/mg was maximum at 30sec, decreasing sharply from 30sec to 10min, more gradually from 10 min to 1 hr, and remaining constant from 1 hr to 3 hr. Both lumbar cord and sciatic nerve showed peak dpm/mg at 2 min post-injection, a sharp decline from 2min to 1 hr, and stabilization from 1 hr to 3 hr. When dpm/mg were analyzed as a % of that of whole blood, both sciatic nerve and lumbar cord showed striking continuous increases from 10 min to 3 hr post-injection. The % of the total radioactivity due to 3H-melatonin was very similar for both sciatic nerve and lumbar cord, averaging 40% at 2 min, 25% at 10 min, and 3% at 2 hr post-injection.

These results show that circulating melatonin is rapidly taken up by mouse sciatic nerve and lumbar cord, and confirm the rapid rate of metabolism of melatonin in the mouse. The continual rise in % blood over time may indicate a possible retention or accumulation of 3H-melatonin and/or its metabolites in sciatic nerve and lumbar cord. Data are currently under analysis to determine whether a selective uptake is present in any of the numerous sites obtained, and whether differences exist between different cord levels, or between different times of day of injection. The typical laboratory mouse may prove to be a useful animal for studying melatonin.

245.1 BETA-ENDORPHIN SUPPRESSION OF SOCIOSEXUAL PROCLIVITY: A TEST OF BEHAVIORAL SPECIFICITY USING A CHOICE PARADIGM. J.B.Wiesner and R.L.Moss. Department of Physiology, University of Texas Health Science Center, Dallas, TX 75235.

Intraventricular administration of beta-endorphin (BE) has been shown to suppress lordosis behavior of rats (Wiesner and Moss, 1982). To test the behavioral specificity of this effect, a four-way choice paradigm was used to assess the preference of experimental female rats for either (1) a stud male, (2) a castrated male, (3) a proestrus female, or (4) an ovariectomized female. The "choice box" apparatus consisted of 4 inner boxes which formed alleys leading from a center box. At the end of each alley was an outer box which held one of the four incentive animals (choices) and which the experimental animal could partially enter. Photocells lining the alleys allowed automated scoring of (1) frequency of entrances into each inner box (IBF); (2) frequency of partial entrances into each outer box (nose poke frequency, NPF); (3) total time spent partially within each outer box (nose poke time, NPT).

At weekly intervals, 14 ovariectomized Sprague-Dawley rats were estrogen-progesterone primed and tested 30 min after intraventricular infusion with saline (1 ul) or BE (1 ug). Subcutaneous treatment of saline or naloxone (2 mg/kg) was administered in conjunction with the intraventricular infusions. The 15 min choice box test was conducted under dim red light; data analysed by 2-way ANOVA (treatment x choice). Overall IBF, NPF, and NPT were significantly greater for the stud male than for each of the other choices ($p<.001$) indicating a clear preference for the stud male. BE failed to alter this preference even though it clearly suppressed receptive and proceptive behavior in other experiments. However, BE significantly lowered total NPF for all incentive animals ($p<.05$); this effect was reversed by naloxone ($p<.05$). The change in total NPF by BE was not due to general behavioral suppression since total IBF (i.e. locomotion) was significantly higher after BE treatment ($p<.005$), an effect also reversed by naloxone ($p<.025$).

The preference for an animal of the opposite sex is consistent with our previous results using this paradigm (Dudley et al.,1983,1984) and reflects sexual motivation as defined by Meyerson and Lindstrom (1973). BE failed to alter this preference, but decreased the tendency to seek general conspecific contact (total NPF); BE may therefore suppress "sociosexual" motivation as a whole rather than sexual motivation specifically. (Supported by NIH grant HD11814.)

245.2 INTRACEREBRAL INFUSIONS OF OXOTREMORINE AFFECT MALE SEX BEHAVIOR. E. M. Hull, D. Bitran*, E. A. Pehek*, R. K. Warner*, and L. C. Band*. Dept. of Psychology, SUNY at Buffalo, Amherst, NY 14226.

Intracerebral infusions of cholinergic agonists facilitate feminine sexual behavior. This facilitation is blocked by peripheral injections of the muscarinic antagonist atropine. We have recently reported that infusions of the cholinergic agonist carbachol into the lateral ventricle of male rats significantly delayed their initiation of sex behavior, but did not alter any other measure. However, carbachol infusions into the preoptic area (POA) produced only a slight delay, and then significantly reduced the number of intromissions required for ejaculation. Since carbachol affects both muscarinic and nicotinic receptors, we wished to test the effectiveness of a more specific muscarinic agonist, oxotremorine (OXO) in producing these effects.

Cannulae were implanted unilaterally into the POA of male Long-Evans rats. OXO (.5, 1, or 2 μg) or normal saline was infused in .5 μl volumes. Tests with a receptive female began immediately and lasted until 30 min after the first intromission. Additional sex behavior tests followed peripheral injections of OXO (16 or 32 μg/kg) or saline.

Compared to the vehicle, OXO (1μg) infusions into the POA significantly reduced the number of intromissions required for the first ejaculation. Peripheral injections of OXO delayed the occurrence of the first intromission, but did not significantly affect other copulatory measures.

These data support our earlier findings of cholinergic inhibition of sexual arousal and a more localized enhancement, within the POA, of a copulatory mechanism. Furthermore, they suggest that muscarinic receptors contribute to both these mechanisms.

245.3 MIDBRAIN INVOLVEMENT IN CHOLINERGIC FACILITATION OF FEMININE SEXUAL BEHAVIOR. G. Richmond* & L.G. Clemens.(SPON:J. Zacks) Michigan State University, E. Lansing, MI 48824.

A central cholinergic mechanism plays an important role in estrogen induction of feminine sexual behavior (lordosis). While the site of this mechanism is not fully identified, we suggest this facilitation results, in part, from activation of muscarine receptors in the midbrain central gray (MCG).

To determine the influence of the MCG on lordosis, ovariectomized female rats (Sherman strain) with lesions of this area were tested for response to muscarine agonists/antagonists. These females were implanted with bilateral cannulae terminating in the ventromedial nucleus of the hypothalamus (VMH) or the lateral ventricles of the brain (LV). Following recovery from surgery, animals were given 3 daily injections of estradiol (EB;.175ug) and tested for lordosis on the fourth day. They then were infused unilaterally with scopolamine (SCOP), a muscarine receptor antagonist (10ug) or vehicle solution. Fifteen min later these females were infused on the contralateral side with .5ug oxotremorine (OXO), a muscarine agonist, or vehicle. Animals were tested for lordosis 5 and 20 min after the last infusion. Females were not receptive following EB treatment alone. While those with MCG lesions displayed some facilitation of lordosis following OXO infusions, this facilitation was significantly smaller than that observed in sham-lesioned females. In both groups, pretreatment with SCOP centrally abolished facilitation. These effects were seen in animals with VMH and LV implants.

In a second part of this study, designed to determine whether additional muscarine receptors, important for lordosis, exist outside the MCG, these same females were treated with EB and progesterone and tested for lordosis. Immediately following this test they were injected with SCOP and retested 45 min later. Both MCG and sham females responded with high levels of lordosis after treatment with the ovarian hormones. However, injection of SCOP resulted in a significant decline in lordosis, and this decline was much greater for females with MCG lesions than for controls.

The MCG thus appears to be an important part of the central cholinergic mechanism mediating feminine sexual behavior. Furthermore, this facilitation seems to be mediated by muscarine receptors.

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245.4 CHOLINERGIC MECHANISMS OF LORDOSIS IN RATS IN THE BASOMEDIAL HYPOTHALAMUS AS REVEALED BY INTRACRANIAL APPLICATION OF SCOPOLAMINE. L.S. Kaufman, D.W. Pfaff, and B.S. McEwen. SPON: R.L. Meisel, Rockefeller University, 1230 York Ave., New York, NY 10021.

It was shown previously (Clemens et al., Pharmac. Biochem. Behav. 13: 81, 1980 & 1980a) that lordosis behavior in rats could be facilitated by cholinergic infusions into the preoptic area or basal hypothalamus. Other work strongly suggests that the ventromedial nucleus (VMN) is the major nucleus that mediates the hormonal aspect of lordosis, and might also be responsible for mediating the cholinergic component of it. The present study was aimed at exploring the mechanisms by which cholinergic activity might influence lordosis.

Nine albino rats were chronically implanted under anesthesia with bilateral cannulae aimed at the VMN. Five days after surgery, estradiol benzoate (E) mixed with cholesterol (1:75) was placed in the cannulae, using a system of removable inner cannulae. Bahavior was tested 3 days later, either in response to stud male rats (lordosis quotient, LQ) or reflex testing (manual stimulation (MS) score). These tests were repeated hourly for 4 hrs. Following this, the inner cannulae were removed and replaced with empty cannulae. A regimen of systemic E plus progesterone (P) was begun, yielding LQs of 50-90. The empty cannulae were then replaced with ones containing scopolamine methyl bromide (either undiluted or diluted 1:10). Rats were tested hourly for 4 hrs and the effects of scopolamine on behavior were assessed. The principal finding was that the 5 rats that responded to intracranial E by increasing their LQs displayed a strong inhibition of lordosis following scopolamine. The percent reduction in all 5 rats was 100%, with latencies of 1-3 hrs. The inhibition lasted throughout the testing session. One rat received a 1:10 dilution of scopolamine and displayed complete recovery after 2 hrs. The above phenomena were elicited in parallel with MS. In 1 rat, however, MS was used alone. In contrast to the above results, rats (4) that did not respond to E also did not respond to intracranial scopolamine. Their mean LQ prior to scopolamine application was 90 and afterwards was 77. These data imply that the inhibitory effect of scopolamine on lordosis may be localized to or near an estrogen sensitive area in the basomedial hypothalamus.

Supported by HD06425 and NS07080.

245.5 CHOLINERGIC FACILITATION OF LORDOSIS IN PROGESTERONE DESENSITIZED FEMALE RATS. P.J. Barr*, T.C. Meyers* and L.G. Clemens. Dept. of Zoology and Neuroscience Program, Michigan State University, E. Lansing, MI 48824.

Estrogen and progesterone regulate the expression of feminine sexual behavior (lordosis) in the laboratory rat. Previous work has shown that central cholinergic activity is important for this behavior. The present experiment examined the relation of cholinergic facilitation to progesterone facilitation of lordosis in the estrogen primed female.

A single, systemic progesterone (P) treatment following estrogen priming will facilitate lordosis; however, a second progesterone treatment 24 hours later is ineffective (P desensitization). To determine whether this failure of a second P treatment to facilitate lordosis reflects a decrease in cholinergic sensitivity, the cholinergic agonist eserine was infused intraventricularly into P desensitized females.

Ovariectomized Sherman strain rats were bilaterally implanted with stainless steel cannulae terminating in the lateral ventricles. In a sequential hormone paradigm 0.25 ug estradiol benzoate (EB) was given daily for 3 days. On day 4 1.5 mg P was given and on day 5 0.5 mg P was administered. In a concurrent paradigm 2.0 ug EB plus 0.5 mg P was given on days 1 through 4 and 0.5 mg P was given on day 5. Tests for lordosis were conducted 4-5 hours after the P treatment on days 4 & 5. Following testing on day 5, females were bilaterally infused with 5 ug eserine or vehicle. This dose of eserine has been shown to facilitate lordosis in estrogen primed female rats. Post infusion tests were conducted 5 and 20 min after eserine administration.

No impairment of eserine facilitation occurred in either the sequential or concurrent paradigm. We suggest that intracerebral cholinergic facilitation of lordosis is independent of the mechanism responsible for progesterone desensitization.

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245.6 ANALYSIS OF THE NICOTINIC CHOLINERGIC FACILITATION OF LORDOSIS. D.R. Weaver* and L.G. Clemens (SPON: J.I. Johnson, Jr.), Dept. of Zoology and Neuroscience Program, Michigan State University, E. Lansing, MI, 48824.

Systemic treatment with nicotine has been shown to facilitate feminine sexual behavior (lordosis) in ovariectomized, estrogen-primed female rats (Fuxe et al., 1977, Pharm. Biochem. Behav. 7; 147-151). This nicotinic facilitation was blocked by the nicotinic receptor antagonist, mecamylamine. The purpose of the present experiment was to determine whether nicotinic receptors are necessary for estrogen plus progesterone induced lordosis. To test this proposal, we examined the effect of mecamylamine treatment on lordosis behavior under two conditions: in females which were showing high levels of sexual receptivity (1) following sequential treatment with estrogen and nicotine and (2) following sequential treatment with estrogen and progesterone (P).

Sexual receptivity was facilitated in ovariectomized, estrogen-primed [.13 ug estradiol benzoate (EB)/day x 3] rats by i.p. nicotine treatment (150 μg/kg, 5 minutes before testing). Mecamylamine treatment (2.5 or 10 mg/kg) administered 30 minutes before nicotine prevented nicotinic facilitation of lordosis. These results agree with those of Fuxe et al., 1977.

In contrast, systemic mecamylamine treatment (5 or 10 mg/kg) did not significantly reduce lordosis in females given EB (0.5 μg/day x 3) plus .5 mg P. Furthermore, bilateral intracerebroventricular infusion of mecamylamine (5 or 10 μg/ .5 μl/ side) also failed to block lordosis in EB plus P primed females.

These results indicate that while mecamylamine (5 or 10 mg/kg) blocked nicotine-induced lordosis behavior, it was not effective in blocking hormonally-induced lordosis. We suggest that nicotinic cholinergic receptors do not play a major role in the estrogen-progesterone induction of sexual receptivity in female rats.

This research was supported by NIH grant HD-06760 to L.G.C. and an NSF pre-doctoral fellowship to D.R.W.

245.7 SEROTONERGIC INHIBITION OF FEMALE RAT SEXUAL BEHAVIOR IN THE BASOMEDIAL HYPOTHALAMUS. J.Moreines, D.Pfaff, and B.McEwen, The Rockefeller Univ., New York, NY 10021.

Optimal sexual receptivity (lordosis) of female rats requires the ovarian secretion of estradiol (E2) followed by progesterone (P). Serotonin is thought to inhibit lordosis at the level of the ventromedial hypothalamus (VMH) (Luine, et al., 1983. Brain Res., 264:344), and P may reverse 5HT inhibition in E2-primed animals (e.g., Espino, et al., 1975. Pharm.Biochem.Behav., 3:557). To test the relations of E2 and P to 5HT, physiological doses of these hormones were administered to ovariectomized females deprived of 5HT following bilateral stereotaxic injections of the neurotoxin, 5,7-dihydroxytryptamine (5,7 DHT) into the region of the VMH. Lordosis frequency (percentage of mounts by sexually vigorous males eliciting a lordotic response), and intensity (0 to 3 representing the absence to maximal dorsiflexion of the spine, respectively) were measured.

We found that the 5,7 DHT injections enhanced the effects of E2 in the absence of P. Doses of E2 sufficient to activate lordosis in 5HT lesioned females were insufficient in sham lesioned controls ($p<.05$). Lordosis frequency was 44% following 5,7 DHT and 9% for shams. However, the intensity of lordosis was less than that normally observed following ovarian E2 and P secretion (Moreines and Powers, 1977. Physiol.Behav., 19:227). Lordosis intensities of 0.6 ± 0.1 and 0.1 ± 0.1 were obtained by 5HT and sham lesioned females, respectively; in contrast, optimal intensity is approximately 2.6.

We then established that following 5HT lesions P continues to promote lordosis and remains obligatory for optimal sexual responding. Both the frequency and intensity of lordosis were elevated for all females when P, but not the vehicle, was administered following E2 ($p<.05$). The frequency was 100% following 5,7 DHT and 75% for shams; the intensity was 2.4 ± 0.3 (5,7 DHT) and 2.2 ± 0.7 (shams).

Our results support the hypothesis that 5HT input at the VMH inhibits hormone actions mediating lordosis. Selective reductions in 5HT potentiated hormone-induced behavior. However, the absence of 5HT from the VMH appears to have a greater influence on E2 sensitivity than on the synergistic effects of P. Whether quantitative reductions in sensitivity to P occur as a result of 5HT lesions is currently under investigation. (Supported by # MH 15125)

245.8 SEROTONIN IN VENTROMEDIAL HYPOTHALAMUS INHIBITS LORDOSIS BEHAVIOR D.L. Allen*, K.J. Renner* and V.N. Luine. Rockefeller University, New York, NY 10021

Increasing evidence suggests that female sexual behavior is regulated by hypothalamic monoaminergic systems. Lordosis responding can be blocked by potentiation of monoaminergic systems with pargyline, an inhibitor of the degradative enzyme monoamine oxidase, placed in the hypothalamus. In this study, pargyline was stereotaxically placed into the hypothalamus and the effects on lordosis were correlated with levels of serotonin (5-HT), 5-hydroxyindole acetic acid (5-HIAA), norepinephrine (NE), and dopamine (DA) in hypothalamic nuclei implicated in the hormonal control of behavior.

Ovariectomized adult female rats were pretreated with 5 ug estradiol benzoate. Two days later they were bilaterally injected with pargyline (35 ug) or saline (0.5 ul) into the ventromedial nucleus of the hypothalamus (VMN). Progesterone (500 ug) was injected subcutaneously 1 hour after the intercranial injection, and sexual behavior was tested 4-5 hours later. Brains were removed and samples of lateral VMN, dorsomedial nucleus (DMN), arcuate-median emminence (ARC-ME), medial preoptic area (POA) and anterior hypothalamus (AH) were dissected using the micropunch technique. These samples were analyzed by HPLC with electrochemical detection.

Intracranial injection of saline (SAL) alone did not affect lordosis quotients (Mean LQ ± SEM: 90 ± 7). Pargyline- treated rats were divided into two groups, one in which sexual behavior was inhibited (INH, Mean LQ 28 ± 7, Range 0 to 40) and the other in which behavior was not inhibited (NI, Mean LQ 96 ± 4, Range 80 to 100). Only 5-HT in VMN and ARC-ME was correlated with the inhibition of lordosis by pargyline. Levels of DA and 5-HIAA were not different between groups for any of the five hypothalamic nuclei analyzed. No differences in monoamine levels between the groups were found in AH or POA. In DMN, 5-HT was increased in both pargyline groups, but they were not different from each other. In ARC-ME, NE was elevated in the NI group, but the INH and SAL groups were similar. In VMN and ARC-ME, levels of 5-HT in SAL and NI were lower than the INH group, but not from each other.

These results provide further evidence of the inhibitory role of 5-HT containing endings in the VMN on hormone dependent lordotic behavior. (Supported by HD12011 and NSF Graduate Fellowship)

245.9 NORADRENERGIC INFLUENCES ON PROGESTIN RECEPTORS AND LORDOSIS IN GUINEA PIGS - CELL NUCLEAR ACCUMULATION OF PROGESTIN RECEPTORS BY NORADRENERGIC INHIBITORS. Jeffrey D. Blaustein. Division of Neuroscience and Behavior, Department of Psychology, Univ. of Mass., Amherst, MA 01003

A series of experiments was performed to study the possible behavioral relevance of the apparent regulation of hypothalamic cytosol progestin receptors by noradrenergic transmission in female guinea pigs. In the first experiment, ovariectomized guinea pigs were injected with estradiol benzoate followed 36 h later by the dopamine-β-hydroxylase inhibitor, U-14,624 or vehicle. Twelve h later, they were injected with progesterone and tested hourly for sexual behavior. Six h after the progesterone injection, a time at which inhibition of sexual behavior by the U-14,624 was confirmed, they were killed, and cytosol and nuclear progestin receptors were assayed in the hypothalamus and cerebral cortex. No difference was seen in the concentration of hypothalamic progestin receptors after drug treatment in these animals that also received a progesterone injection. In subsequent experiments, it was confirmed that the U-14,624-inhibition of progesterone-facilitated sexual behavior is not accompanied by the expected inhibition of nuclear progestin receptor accumulation. The previously reported decrease in hypothalamic cytosol progestin receptors by U-14,624 prior to progesterone injection was confirmed. However, this decrease was accompanied by an increase in the concentration of high affinity, nuclear progestin receptors by 6 h after drug injection. The increase in nuclear progestin receptors was also seen after treatment with the α-adrenergic antagonist, prazosin. U-14,624 does not compete with [^{3}H]R 5020 for binding to the progestin receptor, suggesting that it does not directly cause translocation of progestin receptors. Also, U-14,624 does not cause release of progesterone from the adrenal gland. The results of these experiments suggest that the decrease in the concentration of cytosol progestin receptors caused by noradrenergic inhibitors in guinea pigs is not due to an interference with the formation of cytosol progestin receptors. Rather, it seems that these drugs, in some way, cause the accumulation of progestin receptors in cell nuclei. Furthermore, the results suggest that the mechanism by which these drugs inhibit sexual behavior may not be by interference with the progestin receptor system.

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245.10 EFFECTS OF TESTOSTERONE ON BIOAMINE METABOLITES IN CSF AND SEXUAL BEHAVIOR IN RHESUS MONKEYS. G.A. Davis, S.M. Pomerantz, G.W. Kraemer, M.M. Roy* and R.W. Goy. Wisconsin Regional Primate Res. Center, Univ. of WI, Madison, WI 53715-1299.

That bioamines play a role in the control of sexual activity is supported by many studies, mostly in the rodent. In order to investigate this role in the primate we utilized the levels of bioamine metabolites in cisternal cerebrospinal fluid (CSF) as indicators of bioaminergic activity in brain. We studied several groups of rhesus monkeys under different reproductive conditions, including intact males (IM, n=7), castrated males (CM, n=8), ovariectomized females (OF, n=5) and ovariectomized female pseudohermaphrodites (OPH, n=9). All groups except IM were given daily injections of testosterone propionate (TP, 2mg/kg). CSF was obtained by cisternal puncture (under ketamine anesthesia) before TP treatment was begun and at two and four weeks thereafter. Metabolite levels were determined by HPLC and were found to range, for the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), around 45 ng/ml and for the dopamine metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), around 3 ng/ml and 200 ng/ml, respectively. To test for sexual behavior each animal was observed weekly in a 15 min session with an estrogen-primed stimulus female.

Before TP treatment was begun a significant difference between groups was found in the levels of DOPAC, HVA and 5-HIAA with IM showing the lowest levels. By the fourth week of treatment, the levels of DOPAC and HVA were significantly ($p<0.001$) decreased (13 to 20%) below pretreatment values in CM and OPH, while in OF and IM there were no significant changes. 5-HIAA levels were significantly ($p<0.001$) decreased (18 to 21%) by the fourth week in all TP treated groups, but showed no change in IM. The gonadectomized groups displayed little or no sexual behavior before TP treatment, but after four weeks of the steroid, there was a significant ($p<0.01$) increase in male courtship behavior in CM and OPH, but not in OF. Sexual behavior was high in IM throughout the experimental period.

These results suggest that changes in dopaminergic activity may be related to the action of TP in increasing male courtship behavior, since those groups, CM and OPH, which showed increased levels of behavior with TP treatment also showed reductions in the levels of dopamine metabolites towards the values seen in IM. TP-induced changes in serotonergic activity may also be related to male sexual behavior, but other physiological actions of TP could be involved as well. (Supported by grants HD14821,MH21312, RR00167.)

245.11 INCREASED SELF-ADMINISTRATION OF COCAINE FOLLOWING HALOPERIDOL IS ATTENUATED BY OVARIECTOMY. D.C.S. Roberts, G. Vickers* and J. Dalton*. Dept. of Psychology, Carleton Univ., Ottawa, Canada, K1S 5B6.

Recent studies have shown that manipulation of female sex-related hormones can affect the behavioral response to dopamine agonists and antagonists. These data indicate that the status of ovarian hormones could influence the extrapyramidal and/or the therapeutic effects of neuroleptic drugs.

We have recently shown that there is an extremely high correlation between the clinical (antipsychotic) dose and the degree to which neuroleptic drugs increase cocaine self-administration (Roberts & Vickers, Psychopharm. 82 (1984) 135). We have suggested that the self-administration procedure may provide a useful screening technique for evaluating the antipsychotic effects of neuroleptic drugs, because it may reflect an action on mesolimbic rather than extrapyramidal dopamine function. Therefore, we sought to investigate whether manipulation of ovarian hormones would influence the effect of haloperidol on cocaine self-administration.

Sexually mature female rats were anaesthetized with pentobarbital, ovariectomized (OVX) or sham operated then implanted with a chronically indwelling intravenous cannula. For 4 hrs/day subjects were given access to a lever which initiated a 4 sec infusion of cocaine (1.5 mg/kg/inj.) on a CRF schedule. Approximately one week after being given access to the drug, the animals displayed a stable pattern of daily cocaine intake. No statistical differences in the rate of self-administration were found between ovariectomized and sham operated animals, nor was the rate of intake affected by administration of estradiol benzoate (EB) (50 ug, s.c. X 3 days).

Haloperidol (0.025 - 0.1 mg/kg) produced a dose-dependent increase in self-administration in the sham operated female rats. This effect was significantly attenuated in the OVX group. To date, we have been unable to reverse this effect with either acute or repeated administration of EB (50 ug/kg X 3 days).

In contrast to the attenuation of the effects of haloperidol seen in the self-administration test, and in agreement with previous reports, we have observed an EB reversible augmentation of the cataleptic effects of a larger dose of haloperidol (1 mg/kg). These data indicate that the factors which govern rate of cocaine self-administration are qualitatively different than those which affect the extrapyramidal response (ie. catalepsy). Inasmuch as the self-administration procedure can predict therapeutic efficacy, these data indicate that the antipsychotic action is sensitive to ovarian function. (Supported by the M.R.C of Canada).

246.1 LOCALIZATION OF THE RESPIRATORY EFFECTS OF TRH IN THE RAT MEDULLA. T.J. McCown, R.A. Mueller, A.C. Towle*, and G.R. Breese. Depts. of Anesthesiology, Psychiatry, Pharmacology, and the Biological Sciences Research Center, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27514.

It has previously been shown that thyrotropin releasing hormone (TRH) induces a shortening of both inspiratory and expiratory (T_i and T_e, respectively) phases of the respiratory cycle. The shortening of T_i is rapid in onset, while the basis for the pronounced tachypnea, a shortening of T_e, is later in onset. Since we have observed many TRH immunoreactive nerve terminals throughout the nucleus tractus solitarius (NTS) and nucleus raphe obscurus (RO), we sought to examine the relationship between TRH localization and the respiratory response to microinjection of TRH in these areas of the medulla. Rats were prepared for TRH immunocytochemistry by acrolen fixation and immunoreactivity was demonstrated using antiserum raised against a TRH-BSA conjugate formed with bis-diazotised benzidine. Generally, TRH reactive cells were found in the lateral and medial portions of the ventral surface of the medulla. Fibers were widely distributed throughout the medulla, and appeared concentrated in the NTS and the midline raphe structures.

For the respiratory studies, rats were implanted stereotaxically with 26 guage stainless steel cannulae which terminated just dorsal to the RO (interaural-4.8,0.0lat,0.5vert, according to the atlas of Paxinos and Watson, 1982). After five days of recovery, the rats were placed in a closed chamber plethysmograph, breathing 0.7% halothane in O_2. Microinjection of 100ng of TRH (0.5µl over 3 min. through a 32 guage injection cannulae) into the RO caused significant decreases in the T_i and the respiratory duty cycle (T_i/T_{total}). Concommitantly, no changes were observed in the respiratory frequency, minute volume, P_aCO_2, blood pressure or heart rate for a 15 min. post-treatment period. Conversely, when TRH(100ng) was microinjected into the central canal, lateral NTS or ambiguous nucleus, no changes were found.

These findings indicate that TRH's action on the T_i appear to be localized to the area around the RO in the medulla where TRH reactive fibers are present, while TRH's effects on T_e most probably occur elsewhere in the brain. (Supported by HL-31424 from the NHLBI and N5-17509)

246.2 INTRAUTERINE POSITION DOES NOT ACCOUNT FOR VARIATION IN THE MORPHOLOGY AND BEHAVIOR OF INBRED MICE. C. Kinsley, J. Broida, L. Ghiraldi, C. Konen, J. Miele, and B. Svare. (SPON: R. Oesterreich). Dept. of Psychology, SUNY-Albany, Albany, N. Y., 12222.

During fetal life in multiple birth species, male and female fetuses develop contiguous to, and are influenced by, steroid secretions of the same and opposite sex. The "intrauterine position (IUP) phenomenon" has been studied in outbred mice and rats and differences in morphology (anogenital distance (AGD)) and behavior (parental care, aggression and sex) have been identified (e.g., vom Saal & Bronson, Science, 208: 597, 1980; vom Saal et al, Science, 220: 1306, 1983; Meisel & Ward, Science, 213: 239, 1981). Because the generality of the above findings in outbred animals has not been examined, we studied the extent to which IUP could account for variability in AGD and parental behavior in male and female mice from the C57BL/6J and DBA/2J inbred lines. In the first experiment, 668 C57BL/6J and 445 DBA/2J fetuses were delivered by caesarean section on the 18th day of gestation. The in utero position (i.e., 2M = fetus surrounded by 2 males; 1M = fetus surrounded by 1 male and 1 female; 0M = fetus surrounded by 2 females), sex, AGD, and body weight of each fetus was recorded. Although variability in AGD of both males and females of each inbred line was as great as that previously reported for outbred mice, IUP did not account for any of the variation in this androgen-dependent morphological indicator. Examination of the same data using the "upstream-downstream (UD)" classification scheme (i.e., fetuses are categorized in relation to the presence or absence of males located caudally in the uterus), also did not account for any of the variation observed in AGD. In the second experiment, 103 C57BL/6J male mice and 150 DBA/2J male mice were caesarean delivered and the intrauterine location of each fetus was noted. The animals were then fostered to recently parturient foster mothers. At 60 days of age, the animals were tested for parental behavior (i.e., 1 hr exposure to a 1-3 day old neonate) and classified as retrieving, ignoring, or killing the pup. Once again, while variation in the parental care of male mice was as extreme as that reported for outbred animals, IUP and UD classification schemes did not account for any of the behavioral variation. The findings suggest that IUP and UD phenomena may be genotype dependent. They further suggest that other extra-organismic factors may modulate morphological and behavioral variation in inbred mice.

246.3 SEXUAL DIFFERENCES AND STEROID-INDUCED CHANGES IN METABOLIC ACTIVITY IN TOADFISH SONIC MUSCLE. K.R. Pennypacker, M.L. Fine and R.R. Mills*. Dept. of Biology, Virginia Commonwealth Univ., Richmond, VA 23284.

The sonic apparatus (swimbladder and sonic muscles) of the oyster toadfish Opsanus tau grows larger in males than in females, as do the sonic motor neurons which innervate the sonic muscles. Since the male is more active in sound production than the female, we hypothesized that sonic muscles of the male are biochemically specialized to perform more work.

In order to (1) categorize the fiber type, (2) test for sexual differences, and (3) examine the endocrine basis for any differences, we measured the activity of two anaerobic enzymes, 3 phosphoglyceraldehyde dehydrogenase (3PG) and lactic dehydrogenase (LDH), and two aerobic enzymes, malate dehydrogenase (MDH) and glutamic oxaloacetic transaminase (GOT).

Mean activity levels for the enzymes from sonic muscles are presented for 6 males and 6 females:

	3PG	LDH	GOT	MDH
M	94.00	0.12	10.04	0.67
F	39.38*	0.00	4.51*	0.05

*p <.05

From this and other data, we conclude that toadfish sonic muscle consists of fast fatigue resistant fibers. High 3PG and low LDH levels indicate a sustained level of glycolysis, with pyruvate shuttled into aerobic metabolism. High GOT and low MDH levels indicate that the alpha ketoglutarate formed from pyruvate is transaminated with aspartate to produce glutamate and oxaloacetate, thus bypassing part of the Kreb's cycle and increasing the rate of metabolism.

The endocrine basis for these sexual differences was examined by implanting steroid pellets into ovariectomized females. Testosterone induced a doubling of 3PG activity ($p < .05$) and dihydrotestosterone induced a four fold increase ($p < .05$) in GOT concentration over controls. The steroids had no effect on LDH and MDH activities. Therefore, the activity of both of the enzymes present in higher concentration in males could be increased by androgen implants.

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246.4 BIDIRECTIONAL EFFECTS OF ESTRUS CYCLE ON CHOICE PERFORMANCE IN STRESSED AND NON-STRESSED RATS. S.M. Ryan and S.F. Maier Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Gonadal hormones have been shown to influence behaviors such as choice learning and avoidance learning in rodents. Stress, which influences the pituitary-adrenal axis, produces learning and motivational deficits on some of these same tasks. Taken together, gonadal hormone conditions during stress as well as stress-induced neural and hormonal changes may be important to successful performance. In female rats, stage of estrus, reactivity to stressful events and proficiency in various tasks are likely to interact. In the following study, performance in a choice-escape task was examined as a function of estrus cyclicity and prior exposure to stress.

Subjects were exposed to inescapable tail shock or were simply restrained. Approximately 24 hr later, rats were tested for choice-escape learning in a Y-shaped maze. In order to determine phase of estrus, a vaginal smear was taken just prior to testing. For each trial, a one-mA shock was delivered through a grid floor in the maze and shock was terminated by turning left into the adjacent maze arm. Only animals judged clearly in proestrus(P), estrus(E), metestrus (M), or diestrus(D) were used. Neither shock nor restraint observably altered estrus cyclicity in an earlier group of rats smeared on both days.

Subjects restrained during D or P and tested during P or E, performed well in the choice task. However, if restraint occurred during E or M, and testing occurred during M or D, subjects made significantly more errors than the D/P and P/E restrained groups. Subjects exposed to inescapable shock during D or P, and tested during P or E, made more errors than did the D/P or P/E restrained groups. In contrast, subjects shocked during E, tested during M, performed slightly better than did the E/M restrained group. The M/D shocked group showed a larger trend toward improved performance over the M/D restrained group.

These data suggest that stage of estrus and the concomitant hormonal environment may influence learning on a cognitive performance task. Further, intense stress 24 hr earlier may interact with these hormonal conditions in a bidirectional fashion to produce a choice deficit or to ameliorate poor performance. Overall, this study illustrates the importance of considering gonadal steroids--and concomitant neural and peripheral changes--in learning and performance proficiency, and susceptibility to stress-induced deficits.

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246.5 HYDROCORTISONE INFLUENCES MEMORY PERFORMANCE OF HUMAN MALES. B. E. Beckwith,* T. Petros, * C. Scaglione* and J. Nelson* (SPON: D. C. Riccio). Psychol. Dept. Univ of North Dakota, Box 7187, Grand Forks, ND 58202.

Several animal studies have indicated that treatment with glucocorticoid hormones has influenced memory processes in infrahuman animals (see Bohus et al. in Ganten and Pfaff [Eds.] Adrenal Actions on Brain, New York: Springer-Verlag, 1982, pp 107-148). However, no studies have explored the possibility that glucorticoid hormones may also influence memory processes in humans. The present study was designed to investigate the effects of hydrocortisone on memory for word lists.

Seventy-five male undergraduates providing voluntary, informed consent were administered either glucose or 5, 10, 20 or 40 mg. of hydrocortisone plus glucose in a clear capsule. Treatment conditions were coded to insure a double-blind procedure. After a 60 min. absorption period subjects were asked to listen to eight 12-word lists presented by means of a tape recorder. Subjects were asked to perform immediate oral recall after each list was presented, disregarding order of input. An ANOVA was performed to evaluate treatment effects on mean proportional recall. The results indicated a significant interaction between dose of hydrocortisone and practice (as indexed by order of list presentation). Whereas all doses facilitated recall during presentation of the initial list, recall of later lists was facilitated by the 40 mg dose and was inhibited by the 5 mg dose. The other doses (20 mg and 10 mg) also facilitated recall of the initial list but had no influence on recall of later lists.

These results suggest that glucocorticoid hormones may influence memory in human subjects in a dose dependent manner. These findings suggest that interpretations of the influence of pituitary-adrenocortical hormones (ACTH and glucocorticoids) and vasopressin (which releases ACTH) need to be made with caution and that the influence of these hormones on memory may be complex.

246.6 THE PITUITARY - THYROID AXIS IN PREMENSTRUAL SYNDROME, N.Brayshaw*, M.S. Gold, Research Facilities, Fair Oaks Hospital, Summit, N.J. 07901

Lethargy, fatigue, increased appetite, sleep disturbance and other symptoms reported in premenstrual syndrome (PMS) are consistent with thyroid hypofunction. However, almost every hormonal system but the thyroid has been studied in PMS patients. Since the thyrotropin releasing hormone (TRH) test has become an essential part in the comprehensive evaluation of the patient with a failing thyroid, its use provides an opportunity to evaluate the thyroid axis in PMS.

After an overnight fast each patient was at rest in bed at 800 h for the administration of TRH at 900 h. Blood was taken at 859 for rT_3U, T_3RIA, T_4, and TSH and at 9:15, 9:30, 10:00, 10:30 for TSH. The difference between the peak and baseline TSH (TSH) was then determined (normal is 7-15 units).

We found an augmented TSH response in 20 consecutive patients, exhibiting PMS, as compared to the absence of such a response in 13 age and sex matched patients who did not complain of PMS. All patients with PMS have a TSH greater than 15 and many are considerably higher. The mean TSH for those with PMS is (TSH=22.3) significantly (p 0.01) higher when compared to matched patients (TSH=9) without PMS.

Hypothyroidism is not an all or none phenomenon. There are grades of thyroid failure. An augmented TSH like that reported here for patients with PMS is found in patients with failing thyroid functions. Previously only those with Grade 1 or overt myxedema were studied in PMS studies. Here, patients with subtle abnormalities in TSH responsivity to TRH, described as Grade 3 or subclinical hypothyroidism, were identified. In these PMS patients 50% were Grade 3 subclinical hypothyroidism, 35% were Grade 2 or mild hypothyroidism; and 15% were Grade 1 or overt hypothyroidism. An additional important subgroup accounting for 35% of all the patients with PMS were those with the disease referred to as symptomless autoimmune thyroiditis (SAT), characterized by an augmented TSH, symptoms of a mood disturbance, and presence of antithyroid antibodies(ATA). It is certainly not "symptomless" in PMS.

Thyroid hypofunction may be an etiology of PMS or a significant subgroup of patients complaining of PMS.

246.7 INHIBITION OF CELL NUCLEAR ANDROGEN RECEPTOR BINDING AND MASCULINE COPULATORY BEHAVIOR BY A NEW ANTIANDROGEN, SCH16423. M.Y.McGinnis*, M.C.Mirth* and A.R.Lumia* (SPON:J.E.Shriver). Dept. of Anatomy, Mount Sinai School of Medicine of the City Univ. of New York, NY, NY 10029.

The antiandrogen, flutamide, does not consistently inhibit copulatory behavior in male rats, nor does it inhibit androgen receptor binding in vitro. This has led to the suggestion that a metabolite, rather than flutamide itself, may be the antiandrogenic agent. We have tested the effects of a flutamide metabolite, SCH16423 (Schering, Corp.), on the display of masculine copulatory behavior and on cell nuclear androgen receptor binding in brain. We assessed the effectiveness of SCH16423 to inhibit both maintenance and restoration of copulation in Long-Evans male rats. To test restoration, animals were castrated 4 weeks prior to sc implantation of 2 10mm testosterone (T)-filled Silastic capsules. Rats received either 15 mg SCH16423 daily or oil vehicle. Post-hormone tests were given on days 4, 7 and 11. Copulatory behavior was virtually eliminated after 11 days of SCH16423 treatment. To test the role of SCH16423 in the maintenance of copulation, T-filled capsules were implanted sc at the time of castration. Animals received daily injections of 0,1,5, or 30 mg SCH16423 starting on the day of castration. Although several parameters of masculine copulatory behavior were affected by SCH16423 treatment during the three post-hormone tests, ejaculation was not prevented by SCH16423. In the biochemical studies, we used an exchange assay to determine the effectiveness of SCH16423 in blocking cell nuclear androgen receptor binding both in vivo and in vitro. For in vivo tests, castrate, T-implanted rats received a single injection of 0, 1, 5, 15 or 30 mg SCH16423 1 hr prior to killing. Results indicate that androgen receptor binding, in combined hypothalamus, preoptic area, amygdala and septum (HPAS) is inhibited at all SCH16423 doses. For in vitro assays samples from HPAS were incubated with 10^{-9}-10^{-6}M dihydrotestosterone (DHT), SCH16423 or flutamide. DHT was a strong competitor and SCH16423 a moderate competitor for androgen receptor binding in vitro. Flutamide was ineffective. These results indicate that SCH16423 effectively reduces masculine copulatory potential, primarily affecting restoration of behavior, and that SCH16423 inhibits cell nuclear androgen receptor binding both in vivo and in vitro. SCH16423 may serve as a valuable tool for the exploration of androgen action in brain.

(Supported by a grant from Mount Sinai Medical Ctr)

246.8 RETENTION OF HYPOTHALAMIC NUCLEAR PROGESTIN RECEPTORS MAY MODULATE HEAT DURATION IN FEMALE GUINEA PIGS. Theodore J. Brown* and Jeffrey D. Blaustein (SPON: Jerrold Meyer). Div. of Neurosci. and Behavior, Univ. of Mass., Amherst, MA 01003

Estradiol (E_2) and progesterone (P) interact to activate a period of sexual receptivity (heat) in female guinea pigs. This activation is thought to be mediated by hypothalamic intracellular progestin receptors (PRs). We have recently suggested that the retention of hypothalamic nuclear-bound PRs after P treatment modulates heat duration in female guinea pigs. Experiments were conducted to investigate further the relationship between heat duration and nuclear PR retention.

In ovariectomized (OVX), hormonally treated guinea pigs, heat termination occurs despite the continued presence of E_2 and P. If heat duration is modulated by nuclear retention of PRs, then receptor levels should decline in the presence of elevated blood E_2 and P levels. A Silastic capsule containing E_2 was inserted (sc) into OVX guinea pigs. Forty-eight h later, a 3 cm P-filled or empty capsule was inserted and hypothalamic cytosol and nuclear PR levels were measured 4, 14, 18, and 20 h later. As predicted, nuclear PR levels approached baseline at about the same time as heat terminated in similarly-treated animals.

In a second experiment, OVX guinea pigs were injected with 10 ug estradiol benzoate and received a 1 cm P capsule or an empty capsule 40 h later. Two h later, the capsules were removed from one-half of the animals, and nuclear PR levels were determined 1, 4, 8, and 16 h after capsule removal. At 4 and 8 h, receptor levels were decreased in animals exposed to capsules for 2 h as compared to levels measured in continuously-exposed animals. By 16 h, receptor levels had returned to baseline in the 2 h exposed group while levels were still slightly elevated in the continuously-exposed group. In a separate experiment, similarly-treated animals were tested hourly for lordosis. Contrary to our prediction based on the results of the PR assays, no difference was observed in the time of heat termination for responding animals. However, fewer animals responded in the group exposed to P capsules for 2 h (42% vs. 63%).

These data further support the hypothesis that nuclear PR retention modulates heat duration. However, the correlation between PR retention and heat duration may not be as tight as originally proposed. Experiments using a progestin antagonist are currently being conducted to investigate this relationship further.

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246.9 BLOCKADE OF PROGESTERONE FACILITATION IN FEMALE HAMSTERS BY ANISOMYCIN IN HYPOTHALAMIC AND MIDBRAIN SITES. E.T. Pleim* and J.F. DeBold. Dept. Psych. Tufts Univ., Medford MA 02155.

The behavioral effects of steroid hormones are thought to be mediated by receptor-induced changes in protein synthesis in central target neurons. Because the time course for progesterone (P) action is so much shorter than that for E (0.5-3 hrs vs 1-2 days) a protein synthesis mechanism for P action has been questioned. However, the protein synthesis inhibitor anisomycin blocks P facilitation of sexual receptivity in estrogen-primed female rats when given systemically, or if it is applied directly to the VMH, but not after POA or midbrain applications, supporting the view that both E and P may have their essential, facilitating effects on female sexual behavior in the same area of the brain. However, the VMH may not be the only sufficient site for P facilitation in other species. Since there are 2 sensitive sites for P action in hamsters: the ventral tegmental area (VTA) and the VMH, we tested these sites, and the mPOA, for sensitivity to anisomycin blockade of P facilitation in estrogen-primed female hamsters.

Female hamsters were stereotaxically implanted with bilateral guide tubes aimed at either the mPOA, VMH, or VTA. One week later, 44 hrs after priming with 10 ug EB (SC) 30 ga inner cannulae containing anisomycin were lowered to the target sites. Then 30 mins later, the females were injected with 500 ug P. Two hrs after P, anisomycin inserts were withdrawn. Four hrs after receiving the P, the females were given a 10 minute test for receptivity in 10 gal. aquaria with sexually active males. The same procedure was repeated one week later, except without anisomycin in the inserts. Only animals which showed sexual receptivity on the test one week after anisomycin were included in the analysis.

Anisomycin implants blocked receptivity in a site-specific fashion but did not have any other observable effects on behavior. Receptivity was inhibited with intracranial anisomycin in 18/19 VMH animals, 12/13 VTA hamsters, but only 2/12 POA implanted hamsters were inhibited. Anisomycin applied to other brain regions did not generally inhibit the response to P. These results are different than those reported for rats in which only VMH anisomycin inhibits P response. This species difference may reflect the added importance of mesencephalic sites of action for P in hamsters. Two sites of P action appear to be essential for facilitation of receptivity in hamsters. This may be related to the rigid, immobile lordosis posture the female hamster assumes when she is receptive.

246.10 GONADAL STEROIDS AND PROTEINS IN THE MEDIAL PREOPTIC (MPO) AND VENTROMEDIAL HYPOTHALAMIC (VMH) NUCLEI OF BOTH SEXES. C.W. Scouten, W.E. Heydorn, G.J. Creed*, C.W. Malsbury and D.M. Jacobowitz. Laboratory of Clinical Science, NIMH, Bethesda, MD 20205.

Several lines of evidence converge on the current view that the effects of gonadal steroids on behavior are mediated by direct effects of these hormones on protein synthesis in neurons of the hypothalamus and limbic system that contain specific receptor proteins. Neurons mediating the effects of androgen on male sexual behavior are believed to be concentrated in the MPO, while those mediating the effects of estrogen-progesterone on female sexual behavior are believed to be concentrated in the lateral VMH.

We compared protein profiles from both the lateral VMH and the MPO of male and female rats sacrificed in steroid states that decide copulatory potential. The MPO and VMH were punched from brains of gonadally intact males, induced-estrous females ($Ovx+E_2+P$), and males and females gonadectomized for one month. Proteins from each nucleus were separated by 2-dimensional gel electrophoresis. The gels were silver stained and about 150 proteins from each nucleus were quantified by computerized optical densitometry.

Sex differences in the levels of 15 proteins in the MPO (6 males high, 9 females high) and 2 proteins in the VMH (1 male high, 1 female high) were detected. Of the 15 sex differences in POM, 7 were eliminated by gonadectomy, 3 were attenuated, and 5 were not affected. In the VMH, 1 was eliminated, 1 attenuated. In all, 14 proteins in the MPO of males (10 reduced, 4 increased by castration), 11 proteins in the MPO of females (9 reduced, 2 increased by ovariectomy), 10 proteins in the VMH of males (2 reduced, 8 increased by castration) and 11 proteins in the VMH of females (4 reduced, 7 increased by ovariectomy) were influenced by gonadectomy. Several interesting patterns were noted. Serum albumin was high in the MPO of intact males as compared to all other groups and high in the VMH of estrous females as compared to ovariectomized females. Three other proteins showed similar reversals of pattern between MPO and VMH. Inclusion of the sexually dimorphic nucleus of the preoptic area may be responsible for the higher incidence of sex differences in protein density in MPO as compared to the VMH. Protein changes in the female MPO following gonadectomy may be a reflection of gonadal steroid effects on maternal behavior and pituitary regulation of gonadotropins. The changes in protein density observed in the VMH following castration may suggest a functional role of gonadal steroids in this nucleus in the male.

246.11 CHANGES IN DORSAL MIDBRAIN SINGLE UNIT EXCITABILITY DUE TO OVARIAN HORMONE ADMINISTRATION IN GOLDEN HAMSTERS. M.D. Havens and J.D. Rose Dept. of Psychol. Univ. of Wyoming, Laramie, Wy 82071

The dorsal midbrain is critical to the neural control of hamster lordosis since bilateral lesions of the deep tectum abolish this behavior. The tectum also contains neurons which respond strongly to lordosis-eliciting types of somatic stimulation. These unit responses are selectively enhanced by administration of a lordosis-inducing injection sequence of estradiol and progesterone (Rose and Bieber, J. Neurophysiol., 1984, 51, 1040-1054). In the present study, we evaluated the possibility of a direct influence of ovarian hormones on the excitability of deep tectal neurons by examining the responses of these cells to orthodromic and antidromic activation by ventral midbrain stimulation. Tectal neurons were recorded from previously-ovariectomized, urethane-anesthetized golden hamsters before and after administration of progesterone in propylene glycol vehicle. The hamsters were either primed with 10 ug estradiol benzoate 40 hr prior to preparation for recording or unprimed. The activity of some single units was recorded both before and after progesterone injection, whereas other cells were recorded either before or after the hormone administration. Progesterone decreased the orthodromic excitability of dorsal midbrain single units in estradiol-primed animals such that the average number of spikes/stimulus was reduced. Progesterone also reduced the antidromic excitability of some tectal neurons, attenuating the soma-dendritic portion of the spike waveform. Other cells showed enhanced excitability in the form of a more rapid rise rate of the antidromic spike waveform. Both of these effects on antidromic spikes occured in the first 30 min following a progesterone injection and were seen whether or not the animal was estradiol-primed. Control injections of propylene glycol vehicle without progesterone did not affect unit orthodromic or antidromic excitability. Thus, it appears that progesterone may affect both transsynaptic and antidromic excitability of dorsal midbrain neurons. However, the neural locus of these hormone effects is uncertain and may involve a remote site of action with secondary effects in the tectum.

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246.12 EFFECTS OF ASYMMETRICAL SEPTAL LESIONS AND FRONTOLATERAL HYPOTHALAMIC KNIFE CUTS ON LORDOSIS BEHAVIOR OF RATS. T.R. King* and D.M. Nance. Dept. of Anatomy, Dalhousie Univ., Halifax, Nova Scotia, B3H 4H7 (Spon: J.A. Armour).

It is believed that two primary control systems regulate female sexual behavior. Electrolytic lesions and knife cut experiments have shown that facilitatory control originates from the medial hypothalamic region whereas inhibitory control is mediated by extrahypothalamic regions such as the septal area. It has been proposed that these two opposing systems exert independent control over female sexual behavior (Yamanouchi, P&B, 25, 1983). To test this hypothesis we have investigated the effects of unilateral hypothalamic deafferentations followed by unilateral electrolytic septal lesions on female sexual behavior. Using an extrudable Halász type knife (1.5 mm radius) ovariectomized female rats were given frontolateral hypothalamic knife cuts on either the left or right side of the brain (FLC-L, FLC-R) or sham surgery. Female sexual behavior was tested twice, first with 2 µg estradiol benzoate (EB) for 3 days and tested on day 4 (EB alone), and then immediately following the test, rats were injected with 0.5 mg of progesterone (P) and tested 4-6 hours later (EB+P). Subsequently, all animals received unilateral electrolytic lesions of the lateral septal area on the right side of the brain. Animals were then retested for female sexual behavior as outlined above (EB alone and EB+P). Rats were then perfused and brains processed for histology. The behavioral data was analyzed by ANOVA with repeated measures and significant findings were as follows: FLC-L and FLC-R groups exhibited levels of lordosis behavior comparable to sham operated rats following both EB alone and EB+P treatments. Following unilateral electrolytic septal lesions there was an increase ($p<0.05$) in lordosis behavior across all groups which was specific to the EB alone test. However, there were no group differences between the animals with ipsilateral vs. contralateral hypothalamic knife cuts and septal lesions and relative to animals with unilateral septal lesions and sham knife cuts. Thus the increase in lordosis behavior associated with septal lesions is independent of the medial hypothalamic connections which mediate facilitatory control over female sexual behavior. These results support the concept that these facilitatory and inhibitory systems exert independent control over female sexual behavior and is not mediated by lateral septal-hypothalamic connections. (Supported by MRC of Canada).

246.13 METHYLTRIENOLONE (R1881) FACILITATES FEMALE MATING BEHAVIOR IN ESTROGEN-PRIMED OVARIECTOMIZED RATS AND HAMSTERS. K.L. Olsen, E.T. Pleim*, C.A. Lisciotto* and J.F. DeBold. Dept. Psychiatry and Behav. Sci., SUNY, Stony Brook, NY 11794; and Dept. of Psychology, Tufts University, Medford, MA 02155

Methyltrienolone (R1881 = 17β-hydroxy-17α-methyl-estra-4,9,11-trien-3-one) has been shown to be a valuable ligand in both behavioral and steroid-receptor binding studies. This synthetic steroid binds with high affinity to androgen receptors, but, unlike testosterone or dihydrotestosterone, it is not readily metabolized. One potential problem in using R1881 as an androgen results from its multiplicity of actions. Progestins, as well as androgens, compete significantly for ^{3}H-R1881 binding in the preoptic-hypothalamic area and pituitary (Olsen and Etgen, Neurosci. Abs., 1983). Since R1881 interacts with neural progestin binding then this synthetic steroid may also have progestin-like behavioral properties. The present study examines the ability of R1881 to synergize with estrogen in facilitating female mating behavior in ovariectomized rats and hamsters.

In estrogen-primed rats, R1881 was more effective than progesterone in stimulating lordotic behavior. As little as 5 ug of R1881 facilitated both receptive and proceptive behaviors when given to females primed 48 and 24 hr earlier with 2 ug of estradiol benzoate (EB). Mean lordosis quotient for this group was 93 ± 5.6. In contrast, 25 ug of progesterone was ineffective in activating female-mating behavior even when the EB priming dose is higher than in the present study. Futhermore, R1881-treated rats began to exhibit some behavior 30 min after injection and the behavioral response was maximal 1.5 hr later.

In estrogen-primed hamsters, R1881 was no more effective than progesterone in activating lordotic behavior, but less effective at inhibiting later receptivity. We found that 100 ug of R1881 both initially facilitated and later inhibited receptivity when given to female hamsters primed 48 hr earlier with 10 ug of EB. While 100 ug of progesterone was also required to facilitate lordosis, only 25 ug of progesterone was necessary to inhibit subsequent receptivity. Hamsters treated with 100 ug R1881 began to exhibit lordosis by 2 hr and the response was maximal by 3 hr.

These data indicate that R1881 can be a behaviorally potent progestin in both species. While rats and hamsters are similar in their responsiveness to progesterone, rats are more sensitive to the progestin-like qualities of R1881 than are hamsters.

246.14 EFFECTS OF THE STEROID ANTAGONIST RU 38486 ON BRAIN CYTOSOL PROGESTIN RECEPTOR BINDING AND PROGESTERONE FACILITATION OF ESTROUS RESPONSIVENESS IN FEMALE RATS. A.M. Etgen and R.J. Barfield. Dept. Biol. Sci., Rutgers Univ., New Brunswick, NJ 08903.

In estrogen-primed female rats progesterone (P) facilitates the appearance of estrous responsiveness via an action on the ventromedial nucleus of the hypothalamus (VMN). In the present study the antiprogestin RU 38486 was implanted into the VMN and other brain regions in order to determine (1) if the compound would interfere with P activation of estrous behavior and (2) if the antagonist would exert its effects at the presumed site of P action and/or at other loci involved in the regulation of estrous behavior. It appears that implants of RU 38486 into the VMN antagonize the effects of systemically administered P on estrous behavior. Preliminary evidence also suggests that the action of P on other brain structures might be necessary for the full expression of estrous responsiveness.

We explored the mechanism of RU 38486 action in brain by determining its ability to interact with hypothalamus-preoptic area (HPOA) steroid receptors. RU 38486 appeared to be a competitive inhibitor of progestin binding to brain cytosol receptors with an apparent affinity equal to that of the potent synthetic progestin R5020. Binding isotherms constructed with increasing concentrations of radiolabeled RU 38486 showed that binding did not saturate until relatively high ligand concentrations (10 nM) were added. The number of RU 38486 binding sites in HPOA cytosols increased approximately 50% in female rats primed with 8 μg of estradiol benzoate. In competition studies, RU 38486 was the most effective competitor for its own binding sites. The progestins R5020 and P also competed for RU 38486 binding but were 4- to 5-fold less effective than RU 38486. The corticoids corticosterone, cortisol, dexoycorticosterone and triamcinolone competed 4-fold less effectively than the progestins. Testosterone and estradiol did not displace radiolabeled RU 38486 except at high concentrations (2-10 μM). Thus RU 38486 appears to bind with highest affinity to HPOA progestin receptors; however, it is apparent that the antagonist binds to other sites (e.g., glucocorticoid receptors) as well. These data suggest that the inhibition of estrous responsiveness results from the compound's interference with progestin binding. Supported by grants MH36041 to AME and HD04484 to RJB. The RU 38486 was a generous gift of Roussel-UCLAF, Romaineville, France.

246.15 INDUCTION OF MALE-TYPICAL AGGRESSION BY ANDROGENS BUT NOT BY ESTROGENS IN ADULT FEMALE MICE. N.G. Simon, Dept. of Psychology, Lehigh Univ., Bethlehem, PA 18015, R.E. Whalen, and M.P. Tate, Dept. of Psychology, Univ. of Calif., Riverside, CA 92521.

A major controversy in neuroendocrinology concerns the role of the aromatized and 5α reduced metabolites of testosterone (T) in the activation of intermale aggression. To further explore this issue, we examined the ability of various androgens and estrogens to induce male-typical aggression in adult female mice, a commonly used model system in aggression research.

CF-1 female mice were maintained according to the "Principles for Use of Animals." They were ovariectomized under ether anesthesia and were given a silastic implant containing either T, dihydrotesterone (DHT), methyltrienolone (R1881, a synthetic androgen), E, diethylstilbestrol (DES, a synthetic estrogen) or oil vehicle. In tests for aggression toward a stimulus male over a 35 day period, only the androgen treatments activated aggression (proportion fighting: T (8/12); DHT (6/12); R1881 (6/12). The estrogen treatments were completely ineffective. Additional comparisons showed that there were no differences among the androgen treatments either in the proportion fighting or in the latency in days for aggression to appear (all groups about 20 days).

Subsequent in vitro and in vivo competition studies established the specificity of the DES and R1881 treatments in mouse brain. DES did not compete for ^{3}H-DHT labeled androgen receptor while R1881 did not displace ^{3}H-DES from estrogen receptor.

The results demonstrate that androgenic stimulation alone is sufficient for the activation of aggression. The data also indicate that arguments for aromatization as the primary neuroendocrine mechanism for the induction of aggression by T require modification.

246.16 NON-AROMATIZABLE ANDROGEN ALTERS SNB MOTONEURONAL MORPHOLOGY IN ADULT RATS. Darrell S. Hall*, Renata B. Fishman and S. Marc Breedlove (SPON: I.Zucker). Department of Psychology, University of California, Berkeley, CA 94720.

The motoneurons of the spinal nucleus of the bulbocavernosus (SNB) innervate the striated perineal muscles bulbocavernosus (BC) and levator ani (LA), which mediate androgen-sensitive penile reflexes important for copulatory plug formation in rats. Both the SNB cells and their target muscles possess androgen receptors and morphologically respond to androgen manipulations in adulthood. In Nissl-stained material, the cross-sectional area of SNB somas and nuclei shrink following castration and this effect can be ameliorated by testosterone propionate treatment. We have found that treatment with the non-aromatizable androgen, dihydrotestosterone (DHT) also masculinizes the structure of SNB motoneurons.

Adult male Sprague-Dawley rats were castrated and implanted s.c. with 3 cm-long Silastic capsules (3.2 mm o.d., 1.6 mm i.d.) filled with either testosterone (T) or DHT (Steraloids). Control groups were sham operated males with blank capsules and castrated males with blank capsules. After 42 days, 8 rats in each group were sacrificed and examined. Castration significantly decreased BC/LA and seminal vesicle (SV) weight, and both androgens counteracted this effect. Injections of DHT augment BC/LA weight more than equal dose injections of T. Therefore the smaller response of BC/LA to DHT than T capsules indicates that DHT was released more slowly than was T. The size of thionin-stained SNB motoneuron somata and nuclei also decreased after castration, and both T and DHT compensated for this effect (means ±SEM):

	SV (g)	BC/LA(g)	SNB soma size	Nuclei (μm^2)
Sham males:	1.7 ±.12	1.5 ±.07	1326.9 ±70.1	204.7 ±11.3
Castrates:	0.1 ±.02	0.5 ±.03	1091.0 ±47.1	181.4 ± 3.5
Cast. +T:	1.7 ±.07	1.5 ±.06	1302.3 ±43.8	214.9 ±11.8
Cast. +DHT:	1.0 ±.07	1.1 ±.06	1220.5 ±32.7	199.4 ±12.8

Having established that DHT can masculinize SNB system morphology, we next attempted to test the hypothesis that T alters SNB structure via conversion to DHT. If so, then inhibition of 5-α-reductase should block the effects of T. The drug 4MA (17β-N,N-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one) is reported to act solely as a reductase inhibitor (Brooks et al., 1983, Endoc. 109, 830) and, as expected, 4-cm silastic capsules of 4MA (Dr.J. Brooks, Merck Inst., Rahway, NJ) inhibited the effect of T capsules on SV weight in castrate males (from 1.73 ±.07 to 1.54 ±.07 g). However, this 4MA regimen also inhibited the effect of DHT upon SV (from 1.0 ±.07 to 0.76 ±.05 g), indicating that the drug interfered with androgen receptor binding. Thus we can not use 4MA to test whether T alters SNB structure via reduction to DHT.

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246.17 EVIDENCE FOR TWO PHASES OF ESTROGEN ACTION IN THE BRAIN. A.S. Clark and E.J. Roy. Psychology Department, University of Illinois, Champaign, Illinois 61820.

The behavioral actions of estrogens (E) are characterized by a long interval between the administration of the hormone and the behavioral response. Two main theories describe the mechanism by which E might act on the CNS during this time to induce female sexual behavior 1) E "triggers" an initial event that results in a chemical cascade and 2) E must be "maintained" for a period during which the hormone is slowly changing the chemistry of the target cell. A third alternative is that two or more processes are sequentially stimulated by the hormone; evidence from our laboratory and others indicates that exposure to E need not be continuous in order for a behavioral response to occur, but can be broken down into two brief periods of stimulation. We have investigated some of the cellular events which accompany the two phases of estrogen action.

A first experiment determined whether the induction of neural progestin receptors (PR) shows the same sensitivity to the temporal pattern of E activity as does the behavioral response. Female rats receiving two separate pulses of 0.5 ug free estradiol (E_2) had increased PR relative to untreated control females. Females who received the same total dose in a single pulse (1.0 ug E_2) had PR levels not significantly different from controls.

We examined the dynamics of the estrogen receptor (ER) system during the two phases of E action, evaluating possible alterations during the first phase which might potentiate subsequent hormone action in the nucleus during the second phase. There did not appear to be any rebound increase in cytosol ER as a consequence of prior E exposure. We saw no change in E affinity for cytosol ER as measured by Scatchard analysis following exposure to E. There was no difference in dissociation rates of hormone from nuclear ER after single vs. separate pulses of E_2. However, the two phases may differ in what has been termed "processing" of receptors. A reduction in total receptor content (cytosol + nuclear) reflects processing of receptors. Female rats receiving two pulses of E_2 12 h apart have reduced total ER after the second pulse compared to females receiving a single pulse of E_2. It may be that two separate pulses of E_2 induce changes in total ER content that are necessary for the display of female sexual behavior.

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246.18 AROMATASE ACTIVITY IN RAT HYPOTHALAMIC AND LIMBIC NUCLEI. C.E. Roselli* and J.A. Resko* (SPON: H.G. Spies). Physiology Dept., Oregon Health Sciences University, Portland, OR 97201 and Reproductive Biology and Behavior, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Conversion of androgen to estrogen in the rat brain is catalyzed by aromatase enzymes. The maximum concentration of these enzymes are found within the hypothalamus and amygdala where they appear to play an important role in the process by which androgens affect both behavior and neuroendocrine function. In the present study, we have quantified the levels of aromatase activity (AA) in twenty nuclei and brain regions of the adult rat brain. Individual nuclei were dissected from 300-µm frozen sections according to the Palkovitz technique. Tissues from three animals were pooled and AA was measured by an in vitro radiometric assay that quantifies the stereospecific production of ^3H_2O from [1β-^{3}H]androstenedione as an index of estrogen formation. We report that AA is heterogeneously distributed within the rat brain. The greatest amounts of activity were found in the bed nucleus (n.) of the stria terminalis (NST) (800 fmol·h^{-1}·mg protein^{-1}) and in the n. medialis (MA) and n. corticalis (CA) amygdaloidis (∿ 700 fmol·h^{-1}·mg protein^{-1}) of the male. There was an evident rostral-caudal and medial-lateral gradient in AA throughout the diencephalon. Activity was high in the n. preopticus periventricularis (PVPO) and n. preopticus medialis (MPN); intermediate in the n. preopticus suprachiasmatica, n. anterior hypothalami (AHT), periventricular anterior hypothalamus (PVAH), and n. ventromedialis (VMN): and low in the arcuate nucleus-median eminence, n. preopticus lateralis, and lateral hypothalamus. Regions devoid of measurable AA included the medial and lateral septum, caudate-putamen, hippocampus, and parietal cortex. In the female, AA was greatest in the MA and CA but consistently low in all other areas. We found that AA in the NST, PVPO, MPN, AHT, and VMN was significantly greater in males than in females. Orchidectomy reduced AA to levels seen in females, and administration of testosterone to castrated males restored AA in these areas. No sex or testosterone-induced differences were observed in any other hypothalamic or amygdaloid nuclei.

Our results provide a quantitative profile of AA in discrete hypothalamic and limbic nuclei of the rat brain, as well as information on the control of AA within these discrete regions. Supported by NIH grant 1-P50-HD-1198.

REGULATION OF AUTONOMIC FUNCTION

247.1 DORSAL HORN NEURONS RECEIVING AFFERENT RENAL INFORMATION. M.M. Knuepfer and L.P. Schramm. Dept. Biomed. Engr., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Electrophysiological studies have demonstrated neurons in the medulla and hypothalamus which receive information from the kidney. Neuroanatomical studies have shown that the majority of primary renal afferents project through dorsal roots to the dorsal horn at T10 to T13. In the present study, we describe some neurophysiological properties of dorsal horn neurons receiving afferent renal information. The C1 to C3 level of the spinal cord was exposed in chloralose-anesthetized, artificially respired rats. At C2, four microelectrodes were placed in the dorsal and ventral white matter in order to stimulate selectively each spinal quadrant. In some rats, the spinal cord was transected 5-10 mm rostrad to the stimulating electrodes. The left renal nerve was exposed and gently placed on bipolar, stainless steel hook electrodes for stimulation of afferents. The lower thoracic spinal cord was exposed for two segments between T10 and L1. We recorded from single neurons in the T11 to T13 dorsal horn using metal microelectrodes (3-5 MΩ). Twenty neurons recorded in 10 spinally-intact rats and ten neurons recorded in 4 spinally-transected rats responded to stimulation of the renal nerves. The response was typically a burst of activity (onset latency = 26 ms, estimated conduction distance = 40 mm). The response was generally similar in rats with intact and transected spinal cords. Spontaneous firing rates of responsive neurons ranged from 0 - 50 Hz. Firing was typically aperiodic and occasionally bursting in both groups. Stimulation of the right ventral quadrant of the cervical spinal cord elicited antidromic action potentials in 23% of the neurons receiving renal input. The antidromic conduction velocity was 15 m/s. In spinally-intact rats, stimulation of other sites in the cervical spinal cord often orthodromically excited bursts of activity in dorsal horn neurons receiving afferent renal information. However, in spinally-transected rats, spinal stimulation never elicited excitation, and inhibition of spontaneous or evoked activity was occasionally observed. The majority of neurons receiving renal input were also responsive to stimulation of the skin on the left flank and hindlimb.

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247.2 **DORSAL MOTOR NUCLEUS OF THE VAGUS CONTROLS GLUCAGON RELEASE** W.B. Laughton*, H.-R. Berthoud, and T.L. Powley (SPON: G. Wasserman). Lab. of Regulatory Psychobiology, Purdue Univ., West Lafayette, IN 47907.

Release of pancreatic glucagon is known to be under vagal control (e.g., Holst et al. Acta Phys. Scand. 111:1, 1981), however the organization of the preganglionic parasympathetic neurons mediating this response is not understood. Although the dorsal motor nucleus of the vagus (DMV) has been identified as one of the two major sites of origin of vagal efferent fibers to the abdomen by both anatomical (Kalia & Mesulam, JCN, 193: 467,. 1980) and physiological (Ionescu et al. Endocrinol. 112:904, 1983) techniques, no studies have been performed to date on the role of the DMV in the control of glucagon release.

Male Sprague-Dawley rats with pre-implanted gastric fistulae were food deprived overnight, anesthetized, and equipped with arterial and venous catheters. Sympathetic responses were controlled with α- and β- blockers. Semi-microelectrodes (<50µ) were introduced into the brainstem regions of the DMV in a pattern intended to yield a systematic map. Electrical stimulation of the medulla (50µA, 50Hz, 1msec, 10min) was carried out, with concomitant monitoring of plasma glucose, insulin, glucagon, gastric acid secretion, heart rate, and blood pressure as described previously (Laughton et al., Neurosci. Abs., 9: 113, 1983).

Stimulation of the DMV (n=12) led to an immediate (<2.5min) increase in IRG from 333 to 669 pg/ml (+101±15%), and levels of the hormone remained elevated throughout the stimulation period. Additionally, stimulation of the region immediately surrounding the DMV (<200µ; n=19) or of the efferent axons of the DMV along their intramedullary course (n=14) also caused significantly increased IRG-levels. Neither control sampling (n=15) nor stimulation of sites >200µ from the DMV (n=20) resulted in significant changes in IRG (+2±3% and +9±8% respectively). On average, plasma insulin and gastric acid secretion were also significantly elevated in these animals following stimulation of the DMV. At individual electrode points within the DMV, however, the three subdiaphragmatic responses were uncorrelated (all r^2 < .16), indicating that induction of one response with the stimulating electrode was not necessarily accompanied by the others. This is consistent with the idea that these responses are independently organized within the DMV. (USPHS grant AM27627.)

247.3 AREA POSTREMA LESIONS AND RESTRICTED FEEDING ATTENUATE SYMPATHOADRENAL HYPERGLYCEMIA ELICITED BY 4TH VENTRICULAR BUT NOT SYSTEMIC GLUCOPRIVATION. F.W. Flynn, T.H. Hyde R.R. Miselis and H.J. Grill. Dept. Psychology and Anatomy, Inst. Neurol. Sci., University of Pennsylvania, Philadelphia, PA 19104.

Caudal brainstem metabolic receptors stimulated by glucoprivic agents provide an afferent limb for the compensatory responses of feeding and sympathoadrenal hyperglycemia. Within the caudal brainstem, the area postrema (AP) has been implicated as a site for this metabolic detection. We report that AP lesions (APX) attenuate the sympathoadrenal hyperglycemic response elicited by 4th ventricular (ICV) 5TG but not that elicited by peripherally administered 2DG.

Rats (N=8) were fitted with 4th ventricular cannulae. Food was removed and tail blood samples were collected prior to the 4th ICV injections, and 0.5, 1, 2, 3, and 6 hr post injection. Before APX, rats were injected 4th ICV saline and 210 ug 5TG. Four rats then received APX and 4 others sham lesions. Sham lesioned rats were yoke-fed to APX rats. Rats were again tested with 4th ICV saline and 210 ug 5TG at 1 and 3 weeks postlesion. Postlesion, 4th ICV 5TG elicited hyperglycemia in both sham and APX rats, p's<.05. The hyperglycemic response elicited in APX rats was, however, of significantly lower amplitude and duration than that of their prelesion level and that of the sham lesioned yoke fed rats. Restricted food access in the nonlesioned sham group also significantly attenuated the hyperglycemic response elicited by 4th ICV 5TG compared to their prelesion free feeding response.

In a second experiment, tail blood samples were collected from rats (N=6) prior to IP injections (saline, 250 mg/kg 2DG, and 500 mg/kg 2DG) and 0.5, 1, 2, 3, and 6 hr following the injection. The AP was then aspirated. One and 3 weeks postlesion, rats were retested with IP 250 mg/kg 2DG and 500 mg/kg 2DG, respectively. Prelesion and postlesion plasma glucose levels following 250 mg/kg 2DG were not significantly different at any time. AP lesions significantly enhanced the hyperglycemic response at 2 hr and 3 hr following IP 500 mg/kg 2DG compared to prelesion levels. Thus, AP lesions disrupt the neural circuitry controlling the compensatory sympathoadrenal hyperglycemic response elicited by the central, but not peripheral, administration of glucoprivic drugs.

247.4 THE FOREBRAIN IS NOT ESSENTIAL FOR SUPPRESSION OF GASTRIC ACID SECRETION FOLLOWING INTRACISTERNAL BOMBESIN IN RATS. M.W. Gunlon and Y. Tache. Center for Ulcer Research and Education, V.A. Med. Ctr.-Wadsworth, Los Angeles, CA 90024, and Dept. Medicine, U.C.L.A., Los Angeles, CA 90024.

Intracisternal (IC) injection of bombesin reliably suppresses gastric acid secretion. Cell bodies and terminals containing bombesin, as well as bombesin receptors, have been identified in the lower brainstem (e.g., nucleus tractus solitarius). This area is involved in the control of autonomic function, and is quite close to the site of IC administration. This experiment tested the possibility that lower brainstem mechanisms might account for some of the suppression of gastric acid secretion seen after IC bombesin.

Male albino rats (170-255 g) were food deprived for 24 h. Under ether, they were placed in a stereotaxic instrument. The skull was trephined (control surgery), and an encephalotome was used to completely transect the brain in the coronal plane at the level of the superior colliculus. An IC injection was given immediately thereafter (0, 30, 100, 300 ng bombesin-14 in 10 ul saline). The pylorus was ligated, and pentagastrin was given to all rats (500 ug/kg, sc). Rats were decapitated 2 h following IC injection.

Bombesin reliably suppressed gastric acid secretion in both transected and control rats. Total acid output was reliably decreased in transected rats by 100 and 300 ng (-78%, -84%;p<.05), but not by 30 ng (-60%). Reliable suppression was seen in control rats after 30 and 300 ng (-91%,-94%;p<.02), but not after 100 ng (-65%). Control and transected rats did not differ in basal acid output (179,173 ueq/2 h). Acid concentration was decreased by bombesin in both control and transected rats (-83%,-43%; both p<.05; 300 ng).

These findings suggest that 1) bombesin terminals and receptors located in the lower brainstem may be involved in the regulation of gastric acid secretion; 2) the forebrain does not contain neural circuitry essential for the suppression of gastric acid secretion by IC bombesin; 3) IC bombesin need not travel through the ventricular system to act in the hypothalamic paraventricular nucleus, a site at which bombesin also suppresses gastric acid secretion; 4) bombemay be involved in the regulation of gastric acid secretion at several levels in the brain.

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247.5 EFFECTS OF GLUCOSE AND AUTONOMIC DRUG TREATMENTS ON INSULIN AND GLUCOSE LEVELS IN CHRONIC DECEREBRATE RATS. H.J. Grill and F.W. Flynn. Dept. Psychology and Inst. Neurol. Sci., University of Pennsylvania, Philadelphia, PA 19104.

Previously, Grill and Berridge (Neurosci. Abst., 7:29, 1981) reported that chronic decerebrate rats were hyperinsulinemic yet normoglycemic. The present experiments in decerebrate rats extend this work by examining whether hyperinsulinemia is a chronic condition and if it can be modified by autonomic drug treatments.

Method: All rats were maintained exclusively on 3 daily, 12 ml tube fed sweetened milk meals. Insulin and glucose levels were monitored prior to and for 3 hrs following: 1. complete supracollicular decerebration (N=7) or sham surgery (N=5); 2. gastric carbohydrate loads (12 ml sweetened milk); 3. gastric glucose loads (1.0 g/kg); 4. intravenous glucose loads (0.75 g/kg); and, 5. IP injections of saline, propranolol (2 mg/kg), phentolamine (5 mg/kg), and atropine (5 mg/kg). Injections were made after an overnight, 17 hr fast. Results: At the time of surgery, decerebration did not significantly affect plasma insulin or glucose levels compared to sham operated control rats. Resting (after an overnight fast) insulin levels of control and decerebrate rats were not significantly different; although decerebrate rats tended to be higher on some days. Resting glucose levels of control and decerebrate rats were not significantly different. Following both the gastric carbohydrate and glucose loads and IV glucose load, plasma insulin and glucose levels were significantly higher in decerebrate than in control rats. In response to autonomic treatments, neither atropine nor propranolol affected plasma insulin or glucose levels in either control or decerebrate rats. Phentolamine stimulated insulin secretion in both groups but significantly moreso in decerebrate rats. These data indicate that compared to the reports of hyperinsulinemia in rats with VMH lesions, hyperinsulinemia in decerebrate rats develops more slowly. The hyperinsulinemia in decerebrate rats is largely a postabsorbtive occurrence; fasting returned insulin levels to normal or near normal levels. Last, tube feeding did not mimic the effects of decerebration on insulin secretion following nutrient loads.

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247.6 CENTRALLY MEDIATED HYPOGLYCEMIC EFFECT OF ENDOTOXIN: INVOLVEMENT OF LIPID A AND ENDORPHINS. S. AMIR and M. HAREL. Dept. Isotope Res., The Weizmann Institute of Science, 76100 Rehovot, Israel

The pathophysiologic effects of endotoxin administration include progressively developing hypoglycemia, systemic hypotension, and death. The central nervous system (CNS) has been implicated in the hypotensive and lethal effects and recent studies have shown that endogenous opiate (endorphin) mechanisms might be involved in these endotoxin-initiated actions. Endorphins have also been implicated in endotoxin hypoglycemia, but the role of the CNS has not been elucidated. In the present study, the intracerebroventricular (ICV) administration of endotoxin (E. coli 055:B5, 2.5 ug) in ICR mice resulted in significant hypoglycemia. The plasma glucose decreased from a level of 189.6+6.9 mg% in control mice (n=14) to 96.1+3.01 mg% in ICV endotoxin treated mice (n=14) within 3 h (p 0.05). This centrally-mediated hypoglycemia persisted for 24 h; by 72 h the plasma glucose increased to a level of 155.1+4.6 mg% (n=10). Modification of the endotoxin molecule by incubating with the cationic antibiotic polymixin B (PMB 10:1), which binds the lipid A region of endotoxin, completely prevented the centrally-mediated hypoglycemia. Moreover, PMB, 25 ug, administered ICV significantly attenuated the hypoglycemic response to intravenous (IV) endotoxin (2.5 ug). In this experiment, the plasma glucose increased from a level of 91.9+3.8 mg% in IV endotoxin-treated mice (n=14) to 147.9+2.1 mg% in IV endotoxin/ICV PMB-treated mice (n=14, p 0.05). Furthermore, the ICV administration of the opiate antagonist naltrexone (25 ug) together with endotoxin (2.5 ug) attenuated the centrally-mediated hypoglycemia (plasma glucose of ICV naltrexone-endotoxin mice was 137.2+1.8 mg% (n=8) 3 h post injection and 156.0+10.62 mg% (n=8) 24 h post injection; p 0.05 from respective ICV endotoxin-treated controls). Finally, ICV injection of 25 ug naltrexone attenuated the hypoglycemic response to IV endotoxin (plasma glucose of ICV naltrexone/IV endotoxin treated mice = 139.5+2.9 mg%, n=10; p 0.05 from IV endotoxin-treated controls). These results suggest the existance of CNS endotoxin sensitive receptors whose outputs influence the peripheral mechanisms (hepatic, pancreatic) involved in regulation of plasma glucose homeostasis. Furthermore, the results implicate lipid A as the critical determinant of the central hypoglycemic effect of endotoxin and suggest that this lipid A-mediated action involves activation of central naltrexone-sensitive endorphin mechanisms.

247.7 EFFECTS OF STIMULATION OF RAT MEDIAL FRONTAL INFRALIMBIC CORTEX ON GASTRIC MOTILITY. E.J. Neafsey and K.M. Hurley*, Dept. Anatomy, Loyola Univ. Med. Centr., Maywood, IL 60153

Our laboratory has recently demonstrated a direct projection from the infralimbic region of rat medial frontal cortex (ILC) to the nucleus of the solitary tract, suggesting this cortical region may modulate vagal nerve activity (Terreberry and Neafsey, Brain Res. 278:245-249, 1983). To verify this relationship, we have studied the effects of electrical stimulation of the ILC on gastric motility in rats (n=14) anesthetized with ketamine HCl. In each animal, a balloon catheter was inserted into the stomach lumen via a small incision in the cardiac region of the stomach and sutured into place. The other end of the catheter was attached to a pressure transducer whose output was displayed on a chart recorder along with the occurrence of stimulation and respiratory activity. Before stimulation began a background rhythm of 5-6 contractions per minute was obtained by inflating the balloon with 2-4cc of water. Stimulation was delivered bilaterally via a pair of parallel, glass insulated tungsten electrodes inserted on either side of the superior sagittal sinus (tip separation 1.5mm). In each experiment 3-5 stimulating tracks were made between 2.5 and 5.0mm rostral to bregma. Stimulation was delivered every .5mm along each track between 2 and 5mm below the cortical surface. The stimulation parameters were 60sec trains of negative, .5 msec pulses at 10Hz, currents <100 μamps.

Of 241 stimulated points, 36% had an effect on gastric motility. This effect was generally inhibitory and took several forms. A reduction in gastric tone was most frequently observed (21%) and could be elicited from a more widespread area of ILC than the other responses. The next most frequently observed effect (6%) was a combined reduction of both gastric tone and the amplitude of contractions. The third category of inhibitory responses was a substantial reduction in only the amplitude of the gastric contractions (6%). The least frequently observed effect was a combined reduction in tone and frequency associated with an increase in amplitude (3%). Since in the rat direct vagal stimulation produces an initial, atropine resistant inhibition of motility followed by a strong increase in the amplitude of gastric contractions, the observed inhibitions following cortical stimulation could well be mediated by the vagus nerves. In addition, stimulation effects were abolished when the vagus nerve was sectioned below the diaphragm. The results suggest the ILC may act as a "visceral motor cortex".

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247.8 SUBCORTICAL PROJECTIONS TO THE AMYGDALOID CENTRAL NUCLEUS IN THE RABBIT. B.S. Kapp, J.S. Schwaber[1] and P.A. Driscoll*, Dept. of Psychology, Univ. of Vermont, Burlington, VT 05405 & [1]Dupont Central Research Neurobiology Group, Glenolden, PA 19036.

We have presented evidence suggesting that the amygdaloid central nucleus (ACE) plays an important role in the acquisition and/or expression of conditioned cardiovascular adjustments in the rabbit during Pavlovian fear conditioning (Kapp et al., 1982), quite possibly via direct projections to cardioregulatory nuclei of the medulla (Schwaber et al., 1982). As one of a series of studies designed to further elucidate the nature of this role, we are investigating the afferentation of the ACE in the rabbit. The present study examined the subcortical afferentation of the ACE using retrograde and anterograde tracing techniques.

New Zealand rabbits received injections of HRP (25-50%; 10-50 nl) or the retrograde fluorescent tracer Bisbenzimide (10%, 20-60 nl) into the ACE. Animals were sacrificed from one to three days following the injection. The HRP-injected brains were processed using the TMB procedure (Mesulam, 1978), while those injected with Bisbenzimide were examined using fluorescence microscopy. Sections throughout the brain were examined for retrogradely-labeled neurons.

The following subcortical regions contained substantial numbers of retrogradely-labeled neurons: the bed nucleus of the stria terminalis, the substantia innominata, the amygdaloid basolateral nucleus, the lateral hypothalamic area, the ventromedial nucleus of the hypothalamus, the thalamic paraventricular and parataenial nuclei, the medial component of the medial geniculate nucleus, the substantia nigra-ventral tegmental area, the locus coeruleus, the medial and lateral parabrachial regions, the mesencephalic and pontine raphe nuclei and the nucleus of the solitary tract/vagal dorsal motor nucleus complex. Anterograde tracing experiments in which HRP or ^{3}H-leucine/proline injections (10-60 nl) were made into most of the above regions resulted in anterogradely-transported label in the ACE, confirming the existence of these subcortical-ACE afferents.

The results demonstrate that the ACE is recipient of projections from a variety of subcortical structures, many of which have been implicated in autonomic function and which possess the potential to influence the activity of the prominent ACE descending projection to cardiovascular/autonomic regulatory nuclei of the dorsal medulla.

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247.9 PARAVENTRICULAR NUCLEUS CONTROL OVER DORSAL VAGAL NEURONS, AND GASTRIC ACID SECRETION. R.C. Rogers and P.J. Kahrilas*, Departments of Physiology, Surgery and Gastroenterology Northwestern Univ. Sch. Med. Chicago, IL 60611

The paraventricular nucleus of the hypothalamus (PVN) has been implicated as an important source of direct control over the dorsal motor nucleu of the vagus (DMN) as well as the nucleus of the solitary tract (NST). Since these structures are known to mediate visceral afferent functions such as gastric acid secretion, it is likely that the PVN may exert control over gastric function by a.) directly activating DMN neurons and b.) changing the gain of vago-vagal reflexes by activating vagal NST cells.

In preliminary electrophysiological studies in the rat, we have established that:

a.) 50% of antidromically identified DMN neurons are excited by PVN microstimulation (6-50 uA, 0.5 hz).

b.) 38% of orthodromically-identified NST neurons are excited by PVN microstimulation (8-50 uA, 0.5 hz).

c.) in studies a and b above, PVN neruons could be found in stimulated regions which were antidromically activated by microstimulation through the NST/DMN recording electrodes. Conduction velocities measured in studies a, b, and c were all approximately 0.4 m/sec.

By using standard in vivo gastric perfusion and H^+ titration methods, we have established that:

a.) PVN microstimulation (50 uA, 20 hz, 1msec, 10 min) provokes acid secretory rates 280% higher than basal rates.

b.) oxytocin micropressure ejected into an identified DMN region known to produce acid secretion when activated (50nL, 0.001 M oxytocin) produced a doubling of H^+ secretion rate. 500nL ejections of saline were without effect.

We tentatively conclude that the PVN can modulate gastric secretion by direct contact with neurons in brainstem areas known to mediate cepahalic and gastric phase secretory events. The candidate transmitter substance oxytocin may be the mediator of PVN effects on gastric function.

This work supported in part by NIADDK grant AM32980 to RCR.

247.10 CONTRIBUTION OF THE CERVICAL SYMPATHETIC CHAIN TO THE GASTRIC AFFERENT INPUT TO THE HYPOTHALAMUS. I. Zarco de Coronado, F.C. Barone and M.J. Wayner. Brain Research Laboratory, University of Syracuse, Syracuse, N.Y. 13210. Dept. of Physiology Medical School, University of México, México, D.F.

Our previous reports indicate that single unit responses of the lateral hypothalamus (LH) to the gastric distension were cancelled by the cervical spinal cord (C 1 level) transection or the periacueductal gray (PAG) electrolitic lesion. Those responses were not eliminated after the bilateral cervical vagus or cervical spinal cord (C 2 level) transections. This study presents evidence that gastric distension effects on the unitary activity of the LH also depend of the integrity of the cervical sympathetic systems.

Male Long Evans rats 16 hrs. food deprived were anesthetized with urethane and prepared for hypothalamic extracelular single neuron recordings. A balloon was positioned in the stomach and was filled with 5-12 ml of water while simultaneously monitoring LH unitary activity. The cervical sympathetic chain was dissected in groups of fibers and refered by thin wire loops. The wire was passed through a glass tube and fixed to the skin. A traction of the loops during the ongoing experiments permited to transect bilaterally the sympathetic fibers.

The changes of the basal unitary activity in the LH during the distension were attenuated or eliminated by the interruption of the cervical sympathetic chain. In addition there were changes in blood pressure during the distension not depending of the mechanical compresion over the splanchnic blood vessels.

247.11 RIGHT AND LEFT SIDED BARORECEPTOR AFFERENTS EACH CONVEY BLOOD PRESSURE INFORMATION TO BOTH SIDES OF CORTEX. E.H. Rubin and B.K. Hartman. Dept. of Psychiatry, Washington University Medical School, St. Louis, MO 63110.

The cerebral extraction fraction of water (Ew) is defined as the fraction of ^{3}H-water extracted from the vascular compartment into brain during a single transit. Ew is assayed by a method involving measurement of the ratio of ^{3}H & ^{14}C in brain after intravenous administration of ^{3}H-water and ^{14}C-butanol to anesthetized, paralyzed, ventilated rats. Changes in Ew occur after various physiological and pharmacologic manipulations and this regulation is dependent on the integrity of the central noradrenergic system.

Acute increases in blood pressure (BP) trigger rapid, neurogenic mediated, decreases in Ew in cortex. Elucidating the anatomy of this response should aid in the understanding of the central noradrenergic system's role in central autonomic regulation and allow for more specific studies of the effects of drugs on the system.

Hypertension (hy) induced decreases in cortical Ew require baroreceptor mediated information conveyed by cranial nerves (CN) IX and X. In order to ascertain if this information is carried ipsilaterally, contralaterally, or bilaterally, the effect of right unilateral, left unilateral or bilateral blockade of CN IX and X was studied. BP was increased 40-50mm Hg by angiotensin II. Injection of 5% lidocaine ointment into both carotid sheaths blocked 64% of the BP-induced changes in Ew. Injecting the left or right carotid sheath alone did not inhibit the response in the cortex. Furthermore, the hy-induced decreases in Ew occurred equally in right and left cortex regardless of which carotid sheath was injected. Importantly, unilateral as well as the bilateral injections increased the heart rate and blocked the hy-induced decrease in heart rate which is seen in control animals.

These data suggest that each side of cortex receives information from both left and right baroreceptors and that the afferents from one side are able to compensate for the loss of information caused by blocking the other side. Our working hypothesis is that peripheral BP information is conveyed to the ipsilateral nucleus tractus solitarius. Information then travels bilaterally over polysynaptic pathways to the locus coeruleus (LC) and then from the LC to the ipsilateral cortex.

247.12 CATAPLEXY RELATED TO BLOOD PRESSURE AND PULSE TRANSIT TIME CHANGES. L. Scrima, P. Hartman*, J. Anderson* and K. Winters*. Neurology, U. of Miami Sch. of Med., Miami FL

Narcoleptics have been reported to have lower than normal blood pressure. This fact coupled with the curious behavioral precipitator of cataplexy, i.e., sudden strong emotion, led to the hypothesis that a sudden increase in blood pressure in narcoleptics triggers cataplexy. Because of the rapid onset and brevity of most cataplexy attacks, cuff measurement of blood pressure is not sensitive enough to obtain readings immediately prior to, during and after an attack. Pulse transit time (PTT) has been reported to be highly correlated with changes in systolic blood pressure, is primarily determined by vasotone and is constantly changing, affecting the continuous baroreceptor feedback to the solitarii nuclei (SN), which are important in the regulation of blood pressure. Hence PTT may detect changes in blood pressure that may be associated with cataplexy.

The present study compares PTT averaged over intervals of at least 5 seconds duration. Thus far, 4 narcoleptic volunteers (2 females & 2 males) participated in this within subjects repeated measurements experiment. In addition to EEG, EOG and chin-EMG, EKG, blood pressure (using a Dynamap, taking a reading at 1 min intervals) and pulse transit time (PTT) were monitored and recorded for an entire afternoon while attempts were made to induce cataplexy (e.g., by joking, debating, etc.). The difference between the cataplexy and pre-cataplexy (equal to cataplexy duration) PTT means is highly significant (paired-t, p .02).

The decrease in PTT during cataplexy implies that an increase in vasotone occurs with cataplexy. These preliminary results appear to support the hypothesis that chronic hypotension is an important factor in understanding the etiology of narcolepsy-cataplexy and that sudden increases in blood pressure trigger cataplexy. One possible etiology is that chronic drowsiness induces hypotension, causing a resetting of the baroreceptors to a lower blood pressure level, rendering them hypersensitive to sudden increases in blood pressure; the baroreceptors then exert stronger than normal influence on the solitarii nuclei. The depressor centers of the solitarii would then generate a large depressor response to lower blood pressure and also could stimulate the REM sleep atonia mechanism via solitarii nuclei collaterals to the gigantocellular tegmental field (GTF) and GTF interconnections with the locus coeruleus. The locus coeruleus has direct afferents to the pressor centers of the solitarii, completing a loop.

247.13 TEMPERATURE-SENSITIVE DORSAL RAPHE NEURONS IN VITRO. C. Larry Keenan and Nai-Shin Chu. Department of Neurology, California College of Medicine, University of California, Irvine, CA 92717.

Core temperature is used clinically as a criterion of health. The study of neural substrates underlying thermostasis is important to gain further understanding of the neuronal mechanisms that regulate temperature centrally and peripherally. A primary extrahypothalamic site thought to function in thermoregulation is the dorsal raphe nucleus (Myers and Waller, 1978). Neurons in the dorsal raphe (DR) have been shown to alter firing rates in response to both peripheral and local temperature changes (Cronin and Baker, 1976; Dickenson,1977; Jahns, 1976). It is not known if these cells are intrinsically temperature-sensitive. Therefore we electrophysiologically studied thermosensitivity of 57 DR neurons in brainstem slices obtained from 46 male, Sprague-Dawley rats. Coronal slices (400 microns thick) were continuously perfused in oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF) (Chu and Keenan, 1983). Spontaneous extracellular action potentials were recorded as temperature was varied from 34 C to 42 C from both warm - and cold-responding cells. Three categories of thermosensitive DR cells were found. Biphasic responding cells comprised 13% of the cells studied. These cells fired maximally or minimally over narrow temperature ranges with bell or inverted bell functions. The majority (61%) of thermosensitive neurons responded to rising temperature with increased firing rates. Decreasing discharge rates in response to increasing temperature were measured in 15% of the cells. Only 11% of the cells studied were found to be thermo-insensitive. The temperature-dependent firing rates of most warm-sensitive cells were best fit by a single exponential. Most cold-responding cells had temperature-dependent rates best fit by sigmoid curves that decayed as temperature was increased. Thermal coefficients and Q_{10}'s are comparable to those obtained from DR thermosensitive neurons in vivo (Cronin and Baker, 1976) and from thermoregulatory hypothalamic neurons both in vivo and in vitro (Boulant 1977; Kelso and Boulant, 1982). This is the first study that has demonstrated that thermosensitivity persists in DR neurons in a deafferented slice preparation. The results suggest that at least some of the neurons may be intrinsically thermosensitive. Considering the proposed thermoregulatory role of the raphe, the question is raised: do the raphe mediate thermoregulatory impairments elicited by many psychoactive drugs in mammals?

247.14 **NEURONAL ACTIVITY IN THE SOLITARY COMPLEX RELATED TO AUTONOMIC FUNCTIONS.** W.D. Barber. Dept. of Anatomy, Coll. of Medicine, Univ. of Arizona, Tucson, Arizona 85724.

This study has characterized a novel specific population of neurons in the solitary complex (nucleus and tractus solitarius) related to autonomic function. This study identified neural units activated by gastric distention providing further insight into the organization of this region of the medulla olbongata in terms of the proximity of neuronal populations which orchestrate certain visceral activities; namely digestive, cardiovascular and respiratory. Neuronal activity synchronized to these visceral functions was often observed, each in a specific location, in a single microelectrode track through the solitary system at the level of the obex. Electrophysiological and labelling studies by others have resulted in characterization of the dorsolateral and ventrolateral subnuclei of the solitary group being referred to respectively as the "cardiovascular" and "respiratory" subnuclei. A new category of a "gastric" subnucleus can now be added. Extracellular recordings from anesthetized cats in this study revealed in order 1) cardiovascular, 2) respiratory and 3) gastric related activity as the microelectrode advanced through the solitary system in a dorsoventral direction. Units related to gastric activity extended medially toward the area postrema. These brainstem units were identified on the basis of discharge patterns which were phase-locked to distention or relaxation of the stomach during phasic distention by an intragastric balloon. The majority of these units burst phasically or showed an increase in discharge frequency during gastric distention. The discharge rate of these units ranged from 3 to 6 Hz. Approximately 10% of the neuronal population surveyed were tonically active and were inhibited by distention of the body of the stomach. The response of these units to sustained gastric distention suggests that they are slowly adapting. Local administration of substances into the stomach via a splenic arterial catheter suggests that these brainstem units respond to gastric chemoreceptors as well as stretch receptors. The functional significance and interactions among these neuronal populations residing in the solitary complex is not known at this time. Their close proximity and common input from vagal afferent fibers, however, suggests that the activity of a given population may also reflect the summation of moment to moment changes in adjacent visceral neuronal populations. (Supported by USPHS grant AM31804.

247.15 CONTROL OF ARTERIAL PRESSURE AND GASTRIC MOTILITY BY THE NUCLEUS TRACTUS SOLITARIUS IN RAT. S.E. Spencer and W.T. Talman. Lab. of Neurobiology, Univ. of Iowa and Veterans Admin. Med. Ctr., Iowa City, IA 52242.

The nucleus tractus solitarius (NTS) is the site of termination of visceral afferents. Its role in the regulation of arterial pressure (AP) has been well described but its role in primary and integrative control of gastric motility is less well understood. We have sought to determine whether selective stimulation of neurons in subnuclear regions of the NTS produces tonic and/or phasic gastric motility changes and whether those changes are independent of changes in AP. Twenty-four anesthetized Sprague Dawley rats were instrumented for intra-arterial recording of AP, delivery of i.v. drugs and recording (from balloon sensors) relative antral (A) and fundal (F) gastric pressure changes (GP). The rats were placed in a stereotaxic frame, paralyzed, and ventilated. The dorsal surface of the brain stem was exposed and microinjections (25nl) of sodium L-glutamate (L-glu; 300 pmoles) or saline vehicle were made through glass micropipettes stereotaxically placed in the ventromedial (vm) or dorsomedial (dm) NTS or surrounding structures. The injection sites marked with fast green were confirmed histologically. In some experiments the spinal cord was transected at C1 or the vagi were transected bilaterally in the neck. L-glu, but not saline, injected into the vm NTS, elicited a fall of GP of 1.2 ± 0.2 cm H_2O (mean ± SEM; N=5; $p < 0.005$) and abolished the baseline (5-6/min) phasic AGP wave. AP was not affected by the vm NTS injection. However, the injection of L-glu, but not saline, into the dm NTS elicited a fall of AP of 36.7 ± 9.3 mmHg from a baseline of 130 ± 6.6 mmHg concomitant with a fall of GP of 0.8 ± 0.2 cm H_2O and loss of phasic AGP waves. C1 transection did not affect the GP changes elicited by dm NTS or vm NTS injection (n=2). Vagotomy abolished the GP changes but not the AP changes produced by injections in either region of the NTS (n=4). Injections into the dorsal motor nucleus of the vagus raised GP without affecting AP; injections into the area postrema and hypoglossal did not affect either. These data suggest that phasic and tonic GP activity is neurally controlled by specific regions of the NTS independent of cardiovascular control, while other subnuclear regions integrate both activities. WTT is an Established Investigator for the American Heart Association. SES is the recipient of a postdoctoral fellowship through NIH HL07121.

247.16 **FUNICULAR TRAJECTORIES AND LIKELY SITES OF TERMINATION OF CAT RAPHESPINAL SYMPATHOINHIBITORY NEURONS.** **S.F. Morrison and G.L. Gebber. Depts. of Pharmacol. and Physiol., Mich. State Univ., E. Lansing, MI 48824.**

We previously identified neurons in the cat medullary raphe nuclei with the following characteristics. 1) They exhibit spontaneous activity correlated to inferior cardiac sympathetic nerve discharge (as demonstrated with spike-triggered averaging). 2) They are excited by raising carotid sinus pressure. 3) They have spinally projecting axons, some of which branch in widely separated thoracic spinal segments. We have classified these raphespinal neurons as sympathoinhibitory and refer to them here as RS neurons.

The present study was initiated to define the funicular trajectories and likely sites of termination of RS neurons. The second thoracic spinal segment was systematically explored for sites from which individual RS neurons could be antidromically activated. Threshold current for antidromic activation and response onset latency were recorded for each stimulation site. The position of the main axon of the neuron was assumed to be at the site in the spinal white matter from which the shortest latency antidromic response was elicited with the lowest threshold current (70±12 μA, n=45). The likely termination site of axonal branches was assumed to be at the point in the spinal gray matter from which the longest latency antidromic response was elicited with the lowest threshold current (89±23 μA, n=24).

The main axons of 20 RS neurons were located in the dorsolateral funiculus while those of 25 RS neurons were found in either the ventral funiculus or the medial portion of the ventrolateral funiculus. Mean conduction velocity for dorsolateral axons was 2.8±0.5 m/s while that for ventrally located axons was 2.1±0.1 m/s. The somata of RS neurons with ventrally located axons were distributed differently from those of neurons with dorsolateral axons. The cell bodies of most RS neurons with ventrally located axons were located in a region 2-4 mm rostral to the obex including the ventral portion of nucleus raphe obscurus and the dorsal portion of nucleus raphe pallidus. The somata of RS neurons with dorsolateral axons were concentrated more ventrally in nucleus raphe pallidus. The axonal branches of RS neurons appeared to terminate 1) ipsilaterally in the intermediolateral nucleus (IML; n=16), 2) contralaterally in IML (n=3), 3) bilaterally in IML (n=3) and 4) medial to IML in the ipsilateral lamina VII (n=2). The latter two patterns were observed only for dorsolateral axons. These results indicate that sympathoinhibitory influences of the medullary raphe nuclei are mediated over two spinal pathways which appear to terminate in the IML and more medial portions of lamina VII. **(Supported by NIH grant HL13187.)**

247.17 PARALLEL EXCITATION OF LOCUS COERULEUS NEURONS AND SPLANCHNIC NERVES DURING NOXIOUS STIMULATION. T.H. Svensson, M. Elam* and P. Thorén*. Departments of Pharmacology and Physiology, Karolinska Institutet and University of Gothenburg, Box 60 400, S-104 01 Stockholm, Sweden.

Single unit recording from neurons in the pontine nucleus locus coeruleus (LC) and multifiber recording of peripheral sympathetic nerve activity (SNA) were simultaneously performed in Sprague-Dawley rats with the objective to study a putative correlation between central and peripheral norepinephrine (NE) neuronal activity during sensory noxious stimulation.

Short-term noxious stimulation (paw pinch) caused, as previously described, a biphasic response in LC activity with a brief excitation followed by a short quiescent period. These changes in LC activity were exactly parallelled by changes in SNA. Prolonged noxious stimulation (thermal stimulation) caused also a parallel increase in central and peripheral NE neuronal activity. Both LC firing rate and SNA returned to base line level when the long-term noxious stimulation was terminated.

In addition, both LC and splanchnic neuronal activities were concomitantly and dose-dependently inhibited by intravenous injection of the α_2-receptor agonist clonidine in small doses. This action was, again, in parallel antagonized by administration of the α_2-receptor antagonist yohimbine.

The present findings together with our previous results underline the notion of a strong similarity in regulation of central NE-LC neurons and peripheral NE-SNA.

(Supported by the Swedish Medical Research Council grants No 4747 and 4764).

247.18 STIMULATION IN THE REGION OF THE SUBFORNICAL ORGAN INFLUENCES THE EXCITABILITY OF HYPOTHALAMIC PARAVENTRICULAR NUCLEUS NEURONS (PVN) PROJECTING TO THE DORSOMEDIAL MEDULLA. A.V. Ferguson, T.A. Day, L.P. Renaud. Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Quebec.

Anatomical and electrophysiological studies have suggested that a group of PVN neurons which project to the dorsomedial medulla are involved in CNS regulation of cardiovascular function. We have recently reported that electrical stimulation in the subfornical organ (SFO) causes rapid onset elevations in blood pressure which can be reduced by lesions of the PVN. These data suggest that SFO efferent projections to PVN neurons which in turn project to brainstem and/or spinal cord regions may mediate these cardiovascular changes.

The work reported here was designed to investigate the effects of electrical stimulation in the region of the SFO on the excitability of neurons in the PVN antidromically identified as projecting to the dorsomedial medulla. Experiments were carried out on anaesthetised male Sprague Dawley rats (150-250g). A concentric bipolar stimulating electrode (tip ring separation <0.5mm) directed towards the SFO was implanted and the medial hypothalamus and medulla were exposed through a ventral approach. A glass coated tungsten monopolar electrode (tip exposure <50μm) was positioned in the dorsomedial medulla for the antidromic activation of PVN neurons projecting to this region. Extracellular action potentials were recorded using glass micropipettes filled with 2.0M NaCl and these signals were fed to an on-line PDP 11/34 computer programmed for spike train analysis. Anatomical locations of electrode sites were verified histologically. The effect of stimulation in the region of the SFO was assessed, using peri-stimulus histograms in 42 such antidromically identified neurons. Of these cells, 24 (57%) increased (latency 73.1 ± 18.6 msec: duration 141.7 ± 27.5 msec) and 2 (5%) decreased their excitability, while 16 (38%) were unaffected following stimulation in the region of the SFO.

These data show that electrical stimulation in the region of the SFO increases the excitability of the majority of PVN cells antidromically identified as projecting to the dorsomedial medulla and therefore support a role for these neurons in the pressor response to SFO stimulation.

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247.19 THE ACTION OF γ_2 MSH, A NATRIURETIC/PRESSOR AGENT, ON MAGNOCELLULAR ACTIVITY. K.A. Gruber, L.D. Mitchell, L.D. Wilkin, M.F. Callahan, and A.K. Johnson. Depts. of Psychology, Pharmacology, and Anatomy; and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

Peptides containing the ACTH $^{4-10}$/ γ MSH $^{3-9}$ sequence have dose dependent pressor activity (Gruber et. al. Hypertension, in Press, 1984). γ_2 MSH is a didecapeptide derived from the 16K N-terminus of pro-opiocortin, and contains the γ MSH $^{3-9}$ sequence.

The activity of immunohistochemically identified magnocellular neurosecretory neurons in the supraoptic nucleus (SON of 6 male Sprague-Dawley rats (350-400 g) was used to determine the efficacy of γ_2 MSH in arousing the hypothalamic cardiovascular axis. Single units were identified by constant latency following stimulation of the neurohypophyseal tract, by the ability of antidromically induced action potentials to follow stimulation of 70-110 Hz and by electrical discrimination to distinguish between induced and spontaneously occurring action potentials. Central blood pressure was monitored through the femoral artery and γ_2 MSH was infused through small bore tubing (PE50) implanted into the bifurcation of the carotid artery. All data was transcribed on both paper and magnetic tape and then analyzed by an Apple II computer. Following experimentation, placement of the recording electrodes were confirmed by vasopressin immunohistochemistry.

When the vehicle of 100 µl dH_2O was infused over a 10 sec. period into the carotid artery, no change in either neural activity or blood pressure was observed. However, a γ_2 MSH increased neural activity and blood pressure in a dose-dependent manner. The smallest dose of 100 ng caused slight increase in neural activity. Infusion of 500 ng caused a 10 fold increase in neural activity with little change in blood pressure. Doses of 1, 2.5, 5, 10, 40 µg increased both frequency of neural activity and blood pressure. Throughout the dose range administered, there was little slowing of heart rate, suggesting an uncoupling of the baroreflex.

We conclude that γ_2 MSH may be a potent modulator of hypothalamic cardiovascular interactions.

247.20 HYPOTHALAMIC NEURAL RESPONSES TO THERMAL STIMULATION OF THE HYPOTHALAMUS AND SPINAL CORD IN UNANESTHETIZED UNRESTRAINED RABBITS. R.L. Gerber*, F.W. Klussmann*, and H.C. Heller*, Dept. of Biological Sciences, Stanford University, Stanford, CA 94305, and Inst. fur Normale und Pathologische Physiologie der Universitat, Koln, FDR.

Current understanding of the influence of hypothalamic, ambient, and spinal temperatures on thermoregulation is primarily based on observations of thermoregulatory effectors in unanesthetized animals. Studies of the characteristics of the neural integrative mechanism, however, rely heavily on data from anesthetized or lightly anesthetized, restrained animals. We know from many studies that spinal information is transmitted to the hypothalamus, and, although thermoeffectors are in some cases preferentially facilitated or inhibited by spinal or preoptic/anterior (POAH) temperature manipulations, the nature of and location of the integration is unclear.

We have conducted experiments in unanesthetized unrestrained rabbits using parylene-coated etched fine-wire microelectrodes developed in our laboratory. Eight adult female New Zealand White rabbits were implanted with U-shaped polyethylene (PE 60) thermodes 15 to 20 cm anteriorly entering the spinal canal at L7-S1. Animals were also implanted with an assembly containing four thermodes and a microdrive allowing access to four regions of the POAH. Six of the animals also received a reentrant tube to allow measurement of spinal canal temperature. As the microdrive was advanced, cells having a signal to noise ratio of $\geq$2:1 were recorded on-line by a computer while POAH and spinal temperatures were manipulated.

Initially, most cells observed had firing rates less than 10 Hz. Alhough most of the animals are still being studied, histology in others indicates that the cells were in the caudal aspect of the anterior hypothalamus (AH). Thirteen of 22 cells in this range were responsive to POAH temperature. Three were responsive to spinal temperature. Of these, one responded in the same direction as to POAH temperature, one responded in the opposite direction, and one was insensitive to POAH temperature. Recordings from more rostral locations are currently taking place, and, of the few cells recorded thus far, the only notable difference is that basal firing rates are 1.5 to 2 times those seen more caudally. Though a high proportion of AH cells responded consistently to POAH temperature, relatively few were responsive to changes in spinal temperature. (Supported by NIH NS16317 to H.C.H. and D.F.G. grant to F.W.K.)

247.21 THE MYOELECTRIC RESPONSES OF THE GASTROINTESTINAL TRACT ASSOCIATED WITH EMESIS IN THE DOG. I.M. Lang, S.K. Sarna* and R.E. Condon*. Depts of Surgery and Physiology, Med. Coll. WI, Milw., WI 53226 and VAMC, Wood, WI 53193.

The myoelectric responses of the gastrointestinal tract associated with emesis have not been completely described. Studies using the cat have not included the stomach, and those using the dog have not included the majority of the small intestine. In addition, prior studies have not compared responses during spontaneous vomiting with drug-induced responses. These studies were conducted to describe the myoelectric responses of the gut from gastric antrum to ileum during spontaneous and apomorphine-induced vomiting. Mongrel dogs of either sex were implanted with bipolar seromuscular electrodes using sterile surgical techniques. Experiments were begun after a two week recovery period. Emesis was initiated using apomorphine (2.5 to 15 µg/kg, i.v.) or was observed spontaneously. The specificity of action of apomorphine was determined using the dopamine antagonists domperidone (0.5 µg/kg, i.v.) or haloperidol (50 µg/kg, i.c.v.). The responses associated with spontaneous vomiting episodes are listed below in order of occurrence: 1) slowing of gastric electrical control activity (ECA), 2) disruption of intestinal ECA, 3) a retrograde myoelectric response (RMR), 4) bursts of electrical response activity (ERA) on a slowed intestinal ECA cycle, and 5) retching and vomitus expulsion. The slowing of gastric and intestinal ECA lasted from 1 to 2 minutes. The RMR began about 200 cm aborad to the ligament of Treitz and traveled to the gastric antrum at 23 cm/s. These responses were similar to those occurring after moderate (5-10 µg/kg) doses of apomorphine. Lower doses (2.5-5 µg/kg) stimulated the gastrointestinal responses only and the lowest dose (2.5 µg/kg) stimulated the gastric response only. Higher doses of apomorphine (10-15 µg/kg) caused repeated vomiting episodes with the gastrointestinal responses evident in the first episode only. Domperidone or haloperidol blocked all responses to apomorphine and vagotomy eliminated the gut responses. The results indicated that 1) spontaneous vomiting was preceded by complex myoelectric events of the gut including retrograde peristalsis; 2) apomorphine-induced responses (5-10 µg/kg, i.v.) were similar to resonses occurring during a spontaneous vomit; 3) high doses of apomorphine (> 10 µg/kg, i.v.) caused non-physiologic responses; 4) the gastrointestinal responses associated with vomiting may be observed independent of retching and vomitus expulsion and are vagally mediated; and 5) all responses stimulated by apomorphine were mediated by dopaminergic receptors probably situated within the chemoreceptive trigger zone.

This research was supported by VA grant #7722-01P and BRSG #202-46-01.

248.1 RETINAL ROD OUTER SEGMENT-DERIVED PHAGOSOMES IN HYPOTHYROID AND HYPERTHYROID RATS. C.H. Gray, M.D. Troy* and D.R. Luis*
Biological Sciences Dept., California Polytechnic State University, San Luis Obispo, CA 93407.

It is well established in many vertebrate species that the distal outer segments of rods are phagocytized by the adjacent retinal pigment epithelium (RPE) as an essential part of the photoreceptor renewal cycle. We have examined the influence of thyroxine on the number and size of photoreceptor-derived phagosomes, indices of retinal pigment epithelial activity, during the usual peak time of outer segment shedding after light onset.

Inbred male albino rats were separated into three groups of nine each: control, thyroxine (T4)-treated, and propylthiouracil (PTU)-treated. Hyperthyroidism was induced by daily injections of T4 (20 µg/100g BW) for 8 days. Other groups were vehicle-injected. Hypothyroidism induction was by 0.1% PTU in drinking water for 31 days. A 12:12 hr light-dark cycle was maintained.

Sacrifice order was rotated to prevent time bias against any treatment group. Fixation in glutaraldehyde-paraformaldehyde followed rapid enucleation under anesthesia. Plastic-embedded posterior retinas were sectioned meridionally on an ultramicrotome at 0.5 µm and stained with toluidine blue.

Phagosomes at least 0.75 µm in the greatest dimension were measured and counted within the RPE cells and among their processes. Results are based on ten oil-immersion microscopic fields per eye examined.

	Phagosomes/100 µm RPE	Phagosome Diameter (µm)
Control	19.8 ± 1.6	1.04 ± 0.02
T4-Treated	11.0 ± 0.7*	1.18 ± 0.04**
PTU-Treated	6.0 ± 0.7*	1.10 ± 0.04

*=p < 0.001 **=p< 0.05

Results suggest that deviation from euthyroidism in either direction inhibits RPE phagocytosis during the usual peak time of outer segment shedding. Inhibition is greater in hypothyroidism. The significantly greater size of phagosomes in hyperthyroid rats did not seem to correlate with outer segment diameter, essentially the same in all three groups.

248.2 EFFECTS OF DOPAMINE AND VIP ON FISH CONE HORIZONTAL CELLS. S.C. Mangel* and J.E. Dowling. The Biological Laboratories, Harvard University, Cambridge, MA 02138.

We have investigated the influence of interplexiform cells of the fish retina upon cone horizontal cells by an analysis of the effects of dopamine (DA), forskolin, haloperidol and vasoactive intestinal peptide (VIP) upon the electrophysiological properties of these cells. Experiments were performed on superfused, whole carp retinas. Test drugs were added to the superfusion medium, while membrane potential and light-evoked responses were monitored with intracellular electrodes.

As reported by others (Laufer et al., 1981; Gerschenfeld et al., 1982), we have found that DA application affects the spatial response properties of cone horizontal cells. That is, DA increases the amplitude of the responses of these cells to small spot stimuli and decreases the amplitude of the responses to larger spot stimuli. The differential effect of DA on the responses to large and small spot stimuli is mimicked by the application of forskolin, a stimulator of adenylate cyclase, and blocked by application of haloperidol, a DA receptor antagonist. These findings suggest that the differential effect of DA on the responses of cone horizontal cells to large and small spot stimuli involves an activation of adenylate cyclase through D1 receptors on the horizontal cells.

We have also found that DA application induces other changes in the responses of cone horizontal cells. In particular, DA affects the response waveform of these cells in that it increases the transient character of the responses to small spot stimuli. In fact, during DA application transient responses occur at smaller response amplitudes than occur during the control situation. In addition, DA application reduces the responses of cone horizontal cells to steady or flickering red light stimuli more than the responses to steady or flickering blue or green light stimuli.

Finally, we have found that VIP does not induce a differential effect on the responses of cone horizontal cells to large and small spot stimuli but does induce increases in cAMP production. Because VIP has also been shown to induce an accumulation of cAMP in isolated horizontal cells (Watling and Dowling, 1983), it is possible that the action of VIP-induced cAMP is different from that of DA-induced cAMP.

248.3 EFFECTS OF DOPAMINE ON TRANSMISSION AT SYNAPSES FORMED BY CHOLINERGIC RETINAL NEURONS. W-H. Tsai and D.G. Puro, Laboratory of Vision Research, National Eye Institute, National Institutes of Health, Bethesda, MD 20205.

The purpose of this study was to investigate the effects of dopamine on the function of synapses formed by cholinergic neurons derived from the rat retina. We used an experimental culture system in which striated muscle cells served as postsynaptic targets for cholinergic neurons of retina. This culture system permitted the physiological monitoring of acetylcholine release at synapses formed by retinal neurons.

The results of our electrophysiological experiments indicate that the microiontophoretic application of dopamine can induce a rapid and profound inhibitory effect on cholinergic transmission at retina-muscle synapses. This inhibitory effect ceases promptly with the termination of dopamine application. Thus, this inhibitory effect of dopamine occurs rapidly and reverses quickly. In addition to an inhibitory action of dopmaine, we also observed a marked facilitating effect of this putative neurotransmitter on stimulus-evoked transmission at retina-muscle synapses. When compared to the time course for the inhibitory effect, the dopamine-mediated facilitation of evoked synaptic transmision had a slower onset and longer lasting duration (10 min or more after the termination of dopamine application). Both the short-term inhibition and long-term facilitation could be blocked by haloperidol. Since dopamine was not found to influence the menbrane potential of muscle cells nor the responses of myotubes to microiontophoretically applied acetylcholine, dopamine appeared to have mediated its effects on cholinergic transmission by affecting retinal neurons.

Our findings are consistent with the hypothesis that the action of dopamine on retinal neurons may include a transient inhibitory effect and a long-term modulatory influence.

248.4 **ELECTROPHYSIOLOGICAL CORRELATES OF DOPAMINE DEPLETION IN FROG RETINA.** **M.C. Citron, L. Erinoff, D. Rickman*. Neurology Research, Childrens Hospital of Los Angeles, Los Angeles, CA 90054**

Frog retinas (Rana pipiens) were selectively depleted of dopamine by injecting 6-hydroxydopamine (6-HDA), 100µg in 5µl saline-ascorbate intravitreally into one eye; vehicle was injected into the other eye as a control. From seven to ten days after injection, eyes were excised and single unit responses or ERGs were recorded from an eyecup preparation. After recording initial electrophysiological responses, 100µM apomorphine was dripped onto the eyecup to examine the effects of this dopamine agonist on these responses.

ERGs were evoked using a bright full field flash of 40 msec duration. Single unit responses were recorded and receptive fields were plotted by presenting flashing spots and moving bars. In addition, a spatiotemporal white noise (STWN) stimulus was applied to the receptive field. This stimulus consisted of a 16 by 16 array of square picture elements (pixels) the intensity of each was independently modulated by a binary, random signal. The first and second order kernels for each pixel or pair of pixels was estimated by crosscorrelation of the response with the intensity of the stimuli.

Retinas injected with 6-HDA had an average dopamine depletion of 90%; serotonin levels were unchanged. All dopamine-depleted retinas had lower amplitude ERGs. An increased latency in the b-wave was observed in depleted retinas, confirming our previous findings (Neuroscience Abstracts, 9, 804, 1983). Apomorphine treatment decreased the latency of the b-wave in both depleted and nondepleted retinas. It also shortened the overall timecourse of the b-wave. This effect was most pronounced in depleted retinas.

Horizontal cell responses from depleted retinas were not different from those recorded in control retinas. Neither the responses to flashes, first order kernels, nor predicted responses based on the kernels was affected. This finding supports the hypothesis that b-wave activity is not generated in the outer retina. Ganglion cell responses are currently being recorded in both depleted and control retinas; differences in responses may yield information regarding specific actions of dopaminergic input to these cells and their role in b-wave activity.

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248.5 SOME EFFECTS OF HALOPERIDOL UPON THE RECEPTIVE FIELD CHARACTERISTICS OF CAT RETINAL GANGLION CELLS. Gregory W. Maguire and D.I. Hamasaki, Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, FL, 33101.

The reputed dopamine specific antagonist, Haloperidol was injected into the vitreous of the intact cat eye, and the receptive field characteristics of photopically adapted retinal ganglion cells were monitored before and after injection by single-unit optic tract recordings.

At the doses of 50ug and 100ug, Haloperidol has its most dramatic affects upon the OFF-center Y-cell. Approximately 30 min post-injection, the time at which the effects are most robust, the maintained activity falls to zero, the OFF-excitation to a flashing spot is longer in latency, more transient, and smaller in amplitude. The peripheral shift response is diminished. These responses recover to normal about four hours post-injection, while some responses recover to supra-normal levels at varying times.

In contradistinction, the receptive field characteristics of the ON-center X-cells are least affected by the Haloperidol injection, with only the sustained portion of the center response diminished. Both the OFF-center X-cells and the ON-center Y-cells exhibit moderate changes in their receptive field characteristics following Haloperidol treatment. The OFF-center X-cells show both a longer latency response to receptive field center stimulation and a decrease in their maintained activity. The ON-center Y-cells show an increase in maintained activity and a reduction in their receptive field center peak firing rate. The receptive field center sizes of all cell types are uneffected by Haloperidol. The receptive field center size was taken as the diameter of a flashing spot of white light which elicited the highest peak firing rate. Some of the effects have been demonstrated to be dose dependent.

The results indicate that the response of the cat's retinal dopaminergic receptors to Haloperidol is complex, and suggests that the dopaminergic amacrine cell makes a substantial contribution to retinal information processing in the photopic state.

248.6 THE EFFECTS OF SEROTONINERGIC DRUGS ON THE RESPONSES OF RABBIT GANGLION CELLS. W. J. Brunken, R. J. Jensen, and N. W. Daw. Dept. of Physiology and Biophysics. Washington University School of Medicine. St Louis, MO. 63110

Indoleamine-accumulating amacrine cells have been identified in a variety of mammalian species. In the rabbit these cells have their processes in Cajal's layer 5 of the inner plexiform layer and synapse predominately on the terminals of bipolar cells. The exact identity of the indoleamine these cells use as a transmitter has not been yet established. Serotonin, which has been identified in non-mammalian retinas, appears to be present in only small quantities in the rabbit retina and demonstration of its synthetic enzymes has not been made. On the other hand serotonin has been reported to affect the excitablity of rabbit ganglion cells (Ames and Pollen '69) Other indoleamine putative neurotransmitter candidates may also emerge: for example melatonin has been shown to affect the release of dopamine in the rabbit retina.

As a first step in trying to elucidate the role of an indole in processing in the rabbit retina, the effects of various serotoninergic agonists and antagonists were monitored. The drugs employed included: the serotonin agonist, 5-methoxy N,N, dimethyltryptamine (5-MDT); serotonin (5-HT) and two antagonists, ketanserin and methysergide. Light-evoked responses of retinal ganglion cells were recorded from either the intact eye in situ or from a perfused in vitro eyecup preparation. In the former experimental setup the drugs were introduced to the blood supply via the carotid artery; whereas in the latter case drugs were supplied in the bathing medium.

Off-transient ganglion cells, particularly the large field unit, constitute the majority of cells from which we have obtained recordings. In these cells 5-MDT has the following effects: 1) reduces or eliminates altogether the on-transient response seen with large spots of light; 2) produces a period of prominent on-inhibition and 3) reduces the spontaneous activity. Thus 5-MDT shifts the response of the cell in favor of the central mechanism. These effects of 5-MDT are reversable in 5-20 min. Ketanserin and methysergide both eliminate the surround responses of off-transient cells and reduce the spontaneous activity. Ketanserin unlike methysergide has a bimodal effect on the center-surround balance. In early phases of drug application (first 5-10 min) ketanserin causes a transient increase in the surround component; followed ultimately be an elimination of this component of the cell's response. In preliminary experiments with 5-HT, little effect of this drug on the center-surround balance could be demonstrated unless high concetrations were achieved. However, spontaneous activity was generally increased. Preliminary data on other cell types (on-transient and on-sustained cells) will also be presented.

248.7 INTERACTIONS BETWEEN ENKEPHALINS AND GAMMA-AMINOBUTYRIC ACID (GABA) IN AN AVIAN RETINA. D.M.K. Lam, Y.Y.T. Su and C.B.Watt*. Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030.

In addition to conventional neurotransmitters such as acetylcholine, dopamine and gamma-aminobutyric acid (GABA), a number of peptide immunoreactive substances have been localized to the vertebrate retina (Brecha, 1983). Utilizing the avian retina as a model system, we have attempted to determine the functional roles for a class of opioid peptides, the enkephalins, in the processing of visual information. On the basis of an earlier discovery that enkephalin inhibits the release of GABA in the goldfish retina (Djamgoz et al., 1981), we proceeded to examine whether similar interactions occur in the chicken retina and if they do, what are the anatomical relationships between the enkephalin and GABA systems.

The effect of opiates on the K^+-stimuated release of preloaded GABA was studied using a method reported previously (Ayoub and Lam, 1984). Met^5-enkephalin inhibited the release of GABA in a dose-responsive manner. This inhibition was mimicked by the enkephalin analogue $D\text{-}Ala^2\text{-}D\text{-}Leu^5$-enkephalin (DADL) and the opioid agonist morphine but was reversed by the opioid antagonist naloxone. The maximal inhibition of ^3H-GABA release by any opiate tested was about 40%.

A light microscopic examination of retinal sections processed sequentially for both enkephalin-immunocytochemistry and ^3H-GABA uptake autoradiography revealed clearly that some amacrine cells contain both enkephalin and GABA, while other amacrine cells contain only enkephalin or GABA. To better understand the mechanisms behind the enkephalin-induced inhibition of GABA release, studies are presently underway at the ultrastructural level which examine the synaptic relationships between those cells that contain either enkephalin, GABA or both enkephalin and GABA.

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248.8 EFFECTS OF GABA TRANSAMINASE INHIBITORS AND BICUCULLINE ON THE TEMPORAL FREQUENCY TRANSFER FUNCTIONS OF HORIZONTAL CELLS IN A CYPRINID FISH RETINA. Deborah J. Prince* and M.B.A. Djamgoz. Dept of Pure and Applied Biology, Imperial College, London S.W.7, U.K.

Electrophysiological, ultrastructural and pharmacological studies strongly suggest that there are inhibitory feedback pathways between photopic, red sensitive $L1/H_1$-type horizontal cells (HCs) and cone photoreceptors in cyprinid fish retinae, and that the transmitter at these synapses is γ-aminobutyric acid (GABA). One of the functions this reciprocal interaction appears to subserve is to enhance the range of frequencies of flickering light that can be signalled by the photoreceptors. In this communication we provide further evidence that augmentation and inhibition of GABA action in the retina by GABA-T inhibitors and bicuculline, respectively, produce electrophysiological effects consistent with this hypothesis. $L1/H_1$ type HCs were impaled in retinae isolated from dark-adapted roach (Rutilus rutilus). Their response to a series of sinusoidally flickering red light stimuli delivered from a stroboscope were measured and the relative response amplitude at a given frequency of stimulation was calculated. Data were obtained in untreated retinae, averaged and compared with measurements taken in the same preparation following drug application. Typically, HC responses elicited by sinusoidally flickering light of increasing frequency in a control retina showed similar amplitudes in the range 1-10 Hz. At higher frequencies of light stimulation a gradual decay in response amplitude was observed. Following atomised applications of 150 µm gabaculine or 150 µM γ-acetylenic GABA a general increase of the effective frequency range was observed. For example, at 25 Hz, HCs in control states of the retina showed average response amplitude reductions of 94% whilst following gabaculine and γ-acetylenic GABA treatment the cells gave response amplitude decreases of only 80% and 50%, respectively. Conversely, GABA antagonism, using 160 µM bicuculline, resulted in a narrowing of the frequency domains of the HCs. For example, at 25 Hz, cells in retinae showing an average response amplitude reduction of 28% in the control state yielded a 94% reduction after bicuculline application. The possibility that this effect might be due to a decrease in the receptive field size of the cells was also investigated. Bicuculline was not found to reduce spatial summation in HCs; in fact, a small but significant enhancement was observed.

248.9 VASOACTIVE INTESTINAL PEPTIDE (VIP) STIMULATES RELEASE OF GABA AND GLUTAMATE FROM MAMMALIAN RETINAL SYNAPTOSOMES. M.H. Makman*, J.F. Cubells, D. Smith*, and B. Dvorkin*. (SPON: E.L. Gardner.) Departments of Biochemistry, Molecular Pharmacology, and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

Retinal amacrine cells of a number of species contain VIP. We have previously reported that bovine, rabbit and rat retina contain a highly active VIP-stimulated adenylate cyclase (AC) (Longshore and Makman, Eur. J. Pharmacol., 70:23, 1981). In bovine retina dopamine (DA)- and VIP-stimulated ACs appear to be located on separate neurons; also VIP does not regulate DA neurons presynaptically (Makman et al., Regulatory Peptides, 6:317,1983). However, the precise location of VIP receptors and/or VIP-stimulated AC in mammalian retina is not known. In the present study we have examined the influence of VIP, related peptides and other transmitters on the in vitro release of labeled glutamate and GABA from prelabeled bovine retinal synaptosomes. Also we have investigated the release of ^{3}H-GABA newly formed from ^{3}H-glutamate. VIP did not affect synaptosomal uptake of ^{14}C-glutamate or ^{3}H-GABA. However, following prelabeling with glutamate or GABA, addition of VIP (10^{-8}-10^{-5} M) resulted in a markedly enhanced (2-4 fold) rate of release of label, chromatographically identified as glutamate or GABA. VIP also stimulated the release of ^{3}H-GABA that had been newly formed from ^{3}H-glutamate during a preincubation period. ^{3}H-GABA release was appreciably enhanced by as little as 10^{-8} M VIP. The structurally related peptides PHI, secretin and glucagon also stimulated ^{3}H-GABA release, with relative potencies resembling those for VIP-stimulated AC activity. The effect of VIP was not additive to a stimulatory effect of 50 mM KCl. The adenosine A_2 receptor agonist N-ethylcarboxamide adenosine was weakly active. DA, norepinephrine, isoproterenol, carbachol and forskolin were inactive. 8-Thiomethyl cyclic AMP stimulated (+66%) but to a lesser extent than VIP. Neither carbachol nor DA altered the effect of VIP. VIP stimulated ^{3}H-GABA release in rat retina, but was without effect in rat striatum or frontal cortex. It is proposed that VIP receptors on retinal GABA neurons function to release GABA by a mechanism that may involve cyclic AMP formation. Also, VIP releases ^{3}H-glutamate either from GABA neurons or alternatively from other neurons or cells capable of accumulating labeled glutamate.

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248.10 EFFECTS OF THE PUTATIVE NEUROTRANSMITTER BLOCKERS, 2-AMINO-4-PHOSPHONOBUTYRATE AND MECAMYLAMINE, ON NEURAL PATHWAYS IN GOLDFISH RETINA. Steven E. Walker* and William K. Stell Lions' Sight Centre, University of Calgary Faculty of Medicine, Calgary Canada T2N 4N1.

Specific blockers of transmission at chemical synapses are important tools for pharmacological analysis of neural pathways. We have applied two familiar putatively postsynaptic blockers to the isolated, superfused goldfish retina while recording ganglion cell (GC) responses with extracellular microelectrodes, using post-stimulus time histograms and dose-response functions to analyze the role of pharmacologically identified receptors in spatial receptive field organization and chromatic pathways.

Two-amino-4-phosphonobutyrate (APB) is said to block retinal ON pathways by acting at sign-inverting synapses of photoreceptors to depolarizing bipolars. We found that 1-10 μM APB blocked all light-evoked responses (ON or OFF, red or green, centre or surround) in red-ON-centre cells but enhanced them in red-OFF-centre cells. Most significantly, APB blocked the green-OFF response of red-ON-centre GCs but did not block the green-ON response of red-OFF-centre cells. These results are consistent with blocking action of APB at all synapses of cones upon depolarizing bipolars, provided that the signal from green-depolarizing bipolars is inverted through one or more additional synapses before the GC.

We also employed the nicotinic antagonist, mecamylamine (MCA) to study cholinergic pathways in the inner plexiform layer. Pure ON- or OFF-centre cells (showing both transient and sustained response components) were affected by MCA only at very high doses (ca. 10^{-4}M), at which an overall depression of activity, probably nonspecific, was observed. In contrast, at a much lower dose (<10^{-5}M) MCA totally abolished light-evoked activity but slightly increased dark activity in an ON-OFF transient cell. This suggests that in the goldfish nicotinic cholinergic pathways are instrumental in generating the responses of ON-OFF GCs but not those of ON- or OFF-centre GCs.

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248.11 EFFECTS OF EXCITATORY AMINO ACID ANTAGONISTS ON AMACRINE AND GANGLION CELLS IN AXOLOTL RETINA. David Dvorak*, (SPON: I.G. Morgan), Dept. of Behavioural Biology, R.S.B.S., Australian National University, Canberra, 2601, Australia

The pharmacology of excitation in the inner retina was investigated by assessing the effects of a range of excitatory amino acid antagonists on the responses of amacrine and ganglion cells in axolotl retina. Intracellular recordings were made in the superfused eyecup preparation and test drugs were bath-applied. Membrane resistance was estimated from the slope of current-voltage relations measured in darkness and during the light response in normal Ringer's and in the presence of each antagonist. Light responses and criteria for cell identification were like those described in mudpuppy (Belgum, J.H., Dvorak, D.R. & McReynolds, J.S., *J.Physiol.* 326: 91-108, 1982 and *J.Physiol.* 340: 599-610, 1983).

The kainate/quisqualate antagonist (+/-)-cis-2,3-piperidine dicarboxylic acid (PDA; 2-5mM) eliminated OFF-responses and reduced sustained excitatory input normally present in darkness. PDA had milder effects on ON-responses. In ON-OFF cells the ON-e.p.s.p. typically became more transient and was reduced 10-40% in amplitude, although it always exceeded action potential threshold. In about one half of the cells tested the net increase in conductance underlying the ON-response was unchanged by PDA. γ-D-glutamylglycine (DGG; 2-5mM) had qualitatively similar but reduced effects, while glutamic acid diethyl ester (GDEE; 5mM) was without effect.

The N-methyl-D-aspartate (NMDA) antagonist, 2-amino-5-phosphonovaleric acid (2-APV; 250μM-1mM) eliminated ON-responses and had little effect on OFF-responses or on membrane potential and conductance in darkness. ON-OFF cells typically had a somewhat more transient OFF-e.p.s.p. in the presence of the drug. Low Mg^{2+}, a second selective blocking agent at the NMDA receptor in other regions of the CNS, was applied in concentrations of 1-50μM and had no effect on membrane potential, conductance or light response of any cell type.

The selective elimination of ON- or OFF-responses reported here can be best explained by the known actions of the antagonists in the outer retina. The study raises some doubts about recent claims that the bipolar cell transmitter is an excitatory amino acid (Slaughter, M.M. & Miller, R.F. *Nature* 303: 537-538, 1983).

248.12 DENDRITIC-FIELD ORIENTATION OF HUMAN RETINAL GANGLION CELLS. K.F.BINMOELLER*, J.DINEEN* AND R.W. RODIECK. Dept of Ophthalmology, University of Washington, Seattle, 98195.

We have studied Golgi-impregnated ganglion cells in flat-mounted human retinas. Several morphologic forms were observed, and we concentrated on a distinguishable subgroup that had thickly branching dendritic fields that stratified within a narrow zone of the inner plexiform layer. A scatter diagram of dendritic-field size vs. eccentricity for cells of this subgroup showed two distinct clusters, such that at any eccentricity the cells had either small or large dendritic fields. Morphologic analysis showed that those with the large fields correspond to those described by Polyak as 'Parasol' cells in Golgi-stained transverse sections of monkey and chimpanzee retinas. Likewise those with small fields correspond to his 'Midget' ganglion cells.

The dendritic-fields of many well-filled human Parasol and Midget ganglion cells tend to be elongated rather than circular. Similar elongation has been described for cat ganglion cells, as discussed below. To quantify this elongation, a simple algorithm was used to calculate the best-fitting ellipse to each dendritic-field outline. The position of each analyzed cell on the flat-mounted retina was mapped to its relative position on the retinal hemisphere and the orientation of the major axis of the ellipse for its dendritic-field was marked at this position. A second algorithm was used to determine the point on this hemisphere at which the major axes of these cells tended to point, using the mean-square error as the minimization criterion.

The long axes of the dendritic fields of Parasol cells tend to point to a location close to the fovea (<10 deg), with a high significance level over randomized orientations. A similar analysis of human Midget ganglion cells showed that they likewise tend to point to a location close to the fovea (<10 deg), but with a lower significance level.

The dendritic fields of most ganglion cells in the cat retina also show a radial orientation, centered at or near the area centralis (Leventhal and Schall, '83), which is also reflected in their receptive field properties (Levick and Thibos, '82).

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248.13 **LIGHT MICROSCOPY OF HRP FILLED CAT RETINAL GANGLION CELLS.** Dennis Dacey* (SPON.:D. Tracey). School of Anatomy, University of New South Wales, Kensington NSW, Australia 2033.

Recent anatomical studies of cat retinal ganglion cells have extended the three group, alpha/beta/gamma classification by recognizing new cell types. However, there is persisting disagreement whether new observations suggest additional, discrete ganglion cell types or are best viewed as variation within the gamma cell class. A major difficulty, for either interpretation, has been in acquiring a detailed description of a large sample of identified ganglion cells. In the present study small, iontophoretic injections of horseradish peroxidase (HRP) were made directly into the retina and the morphology of HRP filled ganglion cells was examined in cobalt enhanced, diaminobenzidine reacted retinal whole mounts. The results demonstrate the dendritic and axon morphology of alpha (Class 1) and beta (Class 2) cells and suggest the presence of three additional ganglion cell types, as follows.

Class 3 cells have discoid, multipolar somata (24-30 µm in diameter). A medium caliber axon (0.8-1.2 µm) typically arises from a primary dendrite, close to the soma. Primary dendrites are smooth, radiate and, in their proximal two thirds, branch sparsely by bifurcating. Distal dendrites branch frequently and bear a complement of elongate, varicose appendages. The dendritic field is large (up to 900 µm in the retinal periphery) and is narrowly stratified in the innermost portion of the inner plexiform layer (IPL). Class 4 cells have spherical or fusiform somata (15-25 µm in diameter). A small caliber axon (0.5-1.0 µm) arises directly from the soma. Proximal dendrites are smooth, meandering and frequently branched. Distal dendrites are varicose, often recurve and sport occasional short knobs or spine-like extensions. The dendritic tree is broadly stratified in either the inner (sublamina b) or the outer (sublamina a) portion of the IPL. The field size is medium (up to 450 µm in the retinal periphery) and is thus about half the size of the Class 3 field at a given eccentricity. Class 5 cells have small, spherical somata (8-15 µm in diameter) and fine caliber axons (0.5 µm or less). The dendrites are extremely thin (less than 1 µm), show dense, intricate branching and are heavily studded with spines, knoblike extrusions and long, branched, leaf-like appendages. The dendritic tree extends throughout the IPL and therefore appears relatively bushy in whole mounts. The field is small (up to 250 µm in the retinal periphery) and is thus about one fourth the size of the Class 3 field at a given retinal eccentricity.

Ganglion cell classes 3, 4 and 5, like Class 1 (alpha) and Class 2 (beta) cells, increase in dendritic field size with increasing distance from the area centralis but, at any given eccentricity, each shows a discrete axon diameter, soma size and dendritic tree size, structure and stratification.

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248.14 DISTRIBUTION OF GANGLION CELLS IN LARVAL LAMPREY RETINA. H. Cain* and K. Rubinson. Dept. of Physiol. Biophys., New York Univ. Med. Ctr., New York, NY 10016.

The tracing of degenerated central projections demonstrated the existance of ganglion cells in the retinas of very young, functionally blind, larval lampreys (Kennedy and Rubinson, 1977). These larvae have a morphologically differentiated central retina and a relatively undifferentiated peripheral retina. Putative ganglion cells can be found in the peripheral retina years before the distal layers differentiate (Rubinson and Cain, 1983; Rubinson and Cain, in prep.). The enucleation technique used in the degeneration studies did not permit identification of the position of the ganglion cells in the retina.

In order to locate the ganglion cells, the optic nerve was cut and a crystal of HRP (Boeringer-Mannheim) was placed between the cut surfaces. After 10 days, the eye was removed and processed according to a TMB procedure.

In group 3 larvae, retrogradely filled ganglion cells were found in the central and peripheral retina. These are the youngest animals in which the ganglion cell layer (GCL) extends all the way to the peripheral edge of the retina while the more distal layers are undifferentiated. Labelled ganglion cells were found in the GCL and in the inner portion of the inner nuclear layer (INL). It should be noted that, in the lamprey, the nerve fiber layer is situated between the displaced ganglion cells (DGCs) of the INL and the orthotopic ganglion cells . The estimated ratio of displaced to orthotopic ganglion cells is 1:3.

The ratio of displaced to orthotopic ganglion cells is surprisingly high with respect to the ratios found in other vertebrates. Possible reasons for this apparently high ratio include: 1) a substantial population of cells of orthotopic ganglion cells may remain unlabelled due to the relatively small size of their axon, 2) some of the DGCs present at this stage may be destined to lose their axons and become amacrine cells (Hinds and Hinds, 1978), 3) cell death may occur within the DGC population, or 4) this high ratio may be characteristic of both larval and adult lamprey retinae.

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248.15 THE NEURONAL LOCALIZATION OF CALCIUM-BINDING PROTEIN IN ROD OR CONE DOMINANT RETINA. K.G. Baimbridge, J.J. Miller and D.B. Farber. Dept. Physiology, UBC, Vancouver, B.C., V6T 1W5 and Jules Stein Eye Institute, UCLA School of Medicine, CA 90024.

Calcium binding protein (CaBP), a neuronal specific cytosolic protein, has been shown to be present in a variety of cell types in the CNS. In view of the well known anatomical and physiological organization of neurons within the retina it was of interest to examine the distribution and localization of CaBP in this structure from rod-dominant (rat, mouse) or cone-dominant (squirrel) species to determine whether this protein was associated with a particular functional population of neurons.

Cryostat sections (6-10 µ) of retinal tissue were cut following fixation in 10% formalin, 2% calcium acetate and stained according to procedures previously outlined using a specific antibody to human cerebellar CaBP (Baimbridge and Miller 1982; Brain Research 245, 223-229).

In the mouse and rat intensely labelled ovoid neurons and their processes corresponding to horizontal cells were present in the distal portion of the inner nuclear layer (INL) close to the outer plexiform layer. Other cell types within the INL were much more lightly stained and were few relative to the total number of cells in this region. In the ganglion cell layer a subpopulation of moderately staining cells was evident. CaBP immunoreactivity was notably absent from the receptor cells and their processes.

Retinal neurons of the squirrel exhibited a different pattern of CaBP-like immunoreactivty. In addition to the dense band of stain in the outer portion of the INL corresponding to horizontal cells, morphologically distinct bipolar and amacrine cells localized to the outer and inner portion of the INL respectively were intensely labelled. Processes of these cells extended into the IPL and formed two distinct and intensely stained bands in laminar 1,2 and 4,5. Ganglion cells exhibited moderate to heavy labelling with a far greater number of positive cells than that observed in either the mouse or rat. CaBP immunoreactivity was totally absent from receptor cells.

The observed differences between labelled neurons in the species examined and the fact that not all neurons of a particular population exhibited CaBP-like immunoreactivity suggests that this protein may be a specific marker for subpopulations of retinal neurons which may be functionally distinct.

248.16 OPIOID PATHWAYS IN THE LARVAL TIGER SALAMANDER RETINA: ANATOMICAL AND ELECTROPHYSIOLOGICAL STUDIES. C. B. Watt*, S.E. Feingold*, S.M. Wu, K.R. Fry and D.M.K. Lam. (Spon. C.D.B. Bridges) Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030.

Light microscopic immunohistochemical analyses revealed two populations of neurons which exhibit enkephalin (enk)-like immunoreactivity. The majority of enk-immunostained cells have somas situated in the inner nuclear layer along the border of the inner plexiform layer and may correspond to type 1 amacrine cells (Wong-Riley, 1974). A small number of enk-stained cell bodies were observed in the ganglion cell layer. Such cells were tentatively identified to be displaced amacrine cells, since enk-immunoreactivity was not observed in the ganglion cell axon layer or in the optic nerve. In the inner plexiform layer enk-staining appeared as punctate deposits in sublamina 1 and as a broader band in sublamima 5 adjacent to the ganglion cell layer.

At the ultrastructural level, enk-immunoreactive processes were observed to participate in several synaptic relationships. In sublamina 1 enk-stained varcosities were most often observed to contact unstained vesicle- or non-vesicle-containing profiles. Occasionally, enk-positive profiles received conventional synapses from unstained vesicle-containing profiles. Enk-stained varicosities in sublamina 5 were most often found to be the presynaptic member of a synaptic arrangement in which the postsynaptic element was unstained and was either a vesicle-filled profile (with or without a synaptic ribbon), a non-vesicle-containing profile or the soma of a cell situated in the ganglion cell layer. Less frequently, enk-stained varicosities were observed to receive either conventional or ribbon synaptic input from other unstained profiles.

Initial electrophysiological studies utilizing intracellular recordings indicate that enkephalins and morphine affect the on-center pathways of ganglion cells in this retina.

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248.17 FMRFamide-IMMUNOREACTIVE TERMINAL NERVE EFFERENT FIBRES AND PANCREATIC POLYPEPTIDE-IMMUNOREACTIVE INTRINSIC NEURONES IN GOLDFISH RETINA. L.E. Muske, W.K. Stell and K.S. Chohan* Department of Anatomy and Lions' Sight Centre, University of Calgary Faculty of Medicine, Calgary, Alberta T2N 4N1.

Recently we have shown that retinal projections from the terminal nerve in goldfish contain one or more peptides immunochemically similar to the molluscan cardioexcitatory peptide H-Phe-Met-Arg-Phe-NH_2, or FMRFamide (Stell et al., 1984 PNAS 81:940). The C-terminal sequence of this peptide resembles that of the 36-residue pancreatic polypeptides (PP), most of which, including the neuropeptide NPY, terminate in -Arg-Tyr-NH_2. Since some antisera to FMRF-amide might recognize this sequence in PP-like peptides, we used immunofluorescence techniques (with appropriate preabsorption controls) to compare the localisations of FMRFamide- and PP-immunoreactive (IR) structures in the goldfish retina and optic nerve. Antiserum to FMRFamide (Dockray, "L135") revealed only efferent fibres in optic nerve and retina, as previously described, whereas antisera to avian PP (Kimmel, "Lance") and NPY (Tirenius, "102B") revealed only retinal neurones with somata in the amacrine cell layer and neurites in strata 1, 3 and 5 of the inner plexiform layer. Antiserum to bovine PP (Chance, "615-R110-146-17") recognized both the efferent FMRFamide-IR fibres and the intrinsic PP-IR neurones.

The objective of our studies is to identify peptides specific to identified neurones and to understand their role in retinal function. Since this goal may be confounded by the presence of multiple chemically similar peptides, we crushed the optic nerve unilaterally to interrupt the transport of terminal nerve peptide(s) into the retina without affecting the peptide content of intrinsic neurones. Immunofluorescent localization revealed a marked loss of FMRFamide-IR peptide in efferent fibres distal to the optic nerve crush (vs. contralateral normal control) beginning 5-13 days after surgery, whereas NPY-IR retinal neurones were unaffected. The goldfish retina-terminal nerve system offers an attractive opportunity to characterize vertebrate FMRFamide- and PP-like peptides and to understand their roles in neural function.

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248.18 CHOLINE ACETYLTRANSFERASE-LIKE IMMUNOREACTIVITY IN THE PIGEON RETINA. K. T. Keyser*, H. J. Karten, M. L. Epstein and C. D. Johnson*. Dept. of Psychiatry, SUNY at Stony Brook, NY 11794; Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

Acetylcholine (ACh) occurs within many nervous system structures, including retina. Previous studies employed a number of techniques for the demonstration of ACh, including acetylcholinesterase staining, autoradiography following ^{3}H-choline uptake and -Bungarotoxin binding.

The recent availability of antisera against choline acetyltransferase (CAT) has allowed us to reexamine the localization of ACh in the pigeon retina in terms of the cells contributing to specific laminae as well as the relationship of CAT reactivity to other putative neurotransmitters in the retina. The antiserum used was a rabbit polyclonal against affinity purified CAT from chicken.

Three types of cells exhibited CAT-like immunoreactivity (CATLI) in the pigeon retina: (1) somata (7um in diameter) that were found in the outer tiers of the amacrine cell layer, (2) smaller cells (6um in diameter) limited to the first tier of amacrine cells at the border of the inner plexiform layer (IPL) (the density of these two cell types together was 2200/mm^2), and (3) cells characterized by small cell bodies (5-6um) found within the ganglion cell layer (GCL). In this case, there were about 1800 cells/mm^2.

Two major bands of CAT-positive processes were observed within the IPL. The outermost band was found in a specific portion of lamina 1 (Cajal's scheme), termed lamina 1c by Karten and Brecha. This band originates from the cells in the amacrine cell layer which, in some cases, could be seen to give rise to large caliber dendrites entering lamina 1c. Rarely, processes were observed passing between laminae 1 and 4. However, the major source of CATLI in lamina 4 appears to be the small cells in the GCL. In a very few instances, we observed a thin band of CATLI in lamina 3 of the IPL. The source of these fibers has not been determined.

The identity of the immunoreactive cells in the GCL is uncertain. CATLI has not been observed in the optic nerve head nor has it been possible to label the small CAT-positive cells by the injection of HRP into the tectum. These data support the suggestion of Baughman & Bader that the CAT-positive cells in the GCL are not ganglion cells but rather displaced amacrine cells whose processes arborize in the retina. Supported by EY04796 (HJK) and AM32978 (MLE).

248.19 CROSS-SPECIES COMPARISON OF RETINAL STRUCTURES USING MONOCLONAL ANTIBODIES. J. C. Blanks,* C. Miller# and S. Benzer*+. Doheny Eye Foundation and Departments of Ophthalmology and Pathology#, USC School of Medicine, Los Angeles, CA, and Division of Biology+, California Institute of Technology, Pasadena, CA.

Monoclonal antibodies (Mabs), raised with Drosophila melangaster head and eyes as immunogens, have been shown to have extensive cross-reactivity with human brain tissue. Both mammalian and Drosophila brains contain so many different structures that it is difficult to make direct correlations between them. However, the Drosophila visual system, like the vertebrate retina, is divided into layers corresponding to photoreceptor structures, their cell bodies and successive ganglion cell and synaptic regions, thus providing a system for cross-species comparison.

Cryostat sections of human, monkey, mouse and fly eyes were stained for immunofluorescence microscopy. All Mabs tested were reactive for fly tissue. A systematic comparison, layer by layer, has been made for each Mab. In all vertebrate species studied, approximately one-half of the Mabs tested showed reactivity. Some Mabs were specific for various substructures in the vertebrate retinas, such as photoreceptor outer segments, inner and outer synaptic layers and glial cells. Some of the Mabs also recognized similar structures in the fly eye. The homologies detected by the Mabs will be described.

These Mabs extend the range of anatomical mapping to unknown molecules, providing new markers for structures, cell types and subcellular elements. We are pursuing the comparison of Drosophila and mammalian nervous system antigens and possible corresponding genetic homologies.

These cross-species investigations approach the question of to what extent new nervous system-specific molecules appear in evolution, and whether conserved molecules are used in the same fashion in various nervous systems.

(This work was supported in part by NIH grants EY00188 and EY03042 [JCB], NSF grant PCM 79-11771 [SB], and the Barbara Vanderbilt Peck Foundation of the Amyotrophic Lateral Sclerosis Society of America, the Muscular Dystrophy Association, and the Hereditary Disease Foundation [CM].)

248.20 MONOCLONAL ANTIBODIES AGAINST RETINAL CELLS IN THE ADULT AND DEVELOPING RABBIT. K.R. Fry, N.X. Chen+*, Y.W. Peng* and D.M.K. Lam, Program in Neuroscience and Cullen Eye Institute, Baylor College of Medicine, Houston, TX 77030 and Zhongshan Medical College, Guangzhou, China+*

The introduction of hybridoma technology has resulted in the production of monoclonal antibodies against many specific cell types. We report here on two new monoclonal antibodies, one which stains ganglion cells and another which stains Muller cells in the rabbit retina. BALB/c mice were immunized with a 50% $(NH_4)_2SO_4$ precipitate of cow brain homogenate. The antibodies obtained were screened using indirect fluorescence of rabbit retinal sections. One of the antibodies, AB5, was observed to stain ganglion cells. Dendritic processes in the inner plexiform layer as well as bundles of axons in the nerve fibre layer stained brightly. AB5 immunostaining of ganglion cells, although weak was first observed at 1 day postnatal and appeared adult-like by 6 days postnatal. The specificity of AB5 for ganglion cells was tested in two days: 1) by an isolated cell preparation, and 2) by a backfill preparation in which a fluorescent dye was injected into the contralateral lateral geniculate nucleus. A second antibody, AC4, stained Muller cells in the rabbit. Immunostained processes extended from the inner to the outer limiting membrane with the cell bodies located in the inner nuclear layer. AC4 immunoreactivity was first observed at approximately 6 days postnatal and appeared adult-like at approximately 12 days postnatal. The specificity of AC4 for Muller cells was tested in an isolated cell preparation. The existence of such markers for ganglion and Muller cells will be invaluable in studies of functional organization of the developing retina and visual system.

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248.21 A MONOCLONAL ANTIBODY THAT RECOGNIZES THE FIBER BASKET OF MULLER CELLS IN THE TIGER SALAMANDER RETINA. M. Wilson and K. S. Steimer*. Department of Zoology, University of California, Davis, CA 95616 and Chiron Corporation, Emeryville, CA 94608.

Balb/c mice were immunized intraperitoneally on day 0 and day 21 with Freund's complete adjuvant and a crude membrane preparation obtained from the neural retinae of tiger salamanders. Mouse splenocytes were fused with the myeloma derived cell line P3X63Ag8653 on day 30 following an intravenous boost with immunogen on day 27. Of the resulting 314 lg producing clones, 27 stable lines made antibodies that showed localized binding within the retina as revealed by an indirect immunoperoxidase assay on lightly fixed frozen sections.

Four monoclonal antibodies (MAbs) recognized Muller cells and 8 recognized receptor cells of which 3 were specific for outer segments. Exclusive binding to the inner plexiform layer was shown by 8 MAbs and a further 2 stained both inner and outer plexiform layers. 9 MAbs stained more than one retinal layer and the majority of these did so in a way that precluded specificity to a single cell type, e.g., binding to both ganglion cell fibers and receptor inner segments. No MAbs were found that exclusively bound to the outer plexiform layer, nor were any found that bound exclusively to cell bodies of inner or outer nuclear layers.

One MAb, 2E10, stained a thin layer just scleral to the external limiting membrane. Electron microscopy showed peroxidase reaction product adjacent to the membranes of fibers comprising the fiber basket found between receptor inner segments. We conclude that 2E10 recognizes an antigen, probably membrane associated, that is found on the basket fibers of Muller cells, but is absent or scarce elsewhere on these cells. The nature of the antigen is presently unknown.

248.22 INTRAOCULAR RETINAL TRANSPLANTS. M. del Cerro, D. M. Gash, G. N. Rao, M. F. D. Notter, S. J. Wiegand and M. Gupta, Center for Brain Research, and Departments of Anatomy and Ophthalmology, University of Rochester School of Medicine, Rochester, New York.

We are performing intraocular retinal transplant experiments in the rat to study with a variety of histological and histochemical methods the histogenetic evolution of fetal retinal transplants, implant-host interactions, and the capability of the implants to migrate and repopulate damaged areas of the host retina. The transplanted retinas, from Long-Evans strain dams, contain a differentiated pigment epithelium and a thick, undifferentiated and mitotically active, neuroblastic mass. Initial results, obtained up to two months after transplantation of retinas into the anterior chamber of adult normal eyes, show light and electron microscopic evidence of tissue growth and migration. Unequivocal evidence of differentiation of pigment epithelium, outer and inner nuclear cells, neurites, and synaptic contacts has been obtained. Vascularization of the transplant by host ocular structures has been observed.

Supported in part by grants from The National Eye Institute (EY02632-06 and EY05262) and The Rochester Eye & Human Parts Bank.

248.23 PHYSIOLOGY AND MORPHOLOGY OF MAMMALIAN RETINAL NEURONS IN DISSOCIATED CELL CULTURE. Hermes H. Yeh, Laboratory of Vision Research, National Eye Institute, NIH, Bethesda, MD 20205.

A long-term cell culture system consisting of dissociated cells from the rat retina has been developed to facilitate characterization of the physiological properties and pharmacological responses of mammalian retinal neurons. The aim of this initial survey was to reveal the morphological features of single, visually-identified retinal neurons maintained in culture, to describe some of the electrical properties of retinal neurons and to examine their sensitivity to certain exogenously-applied putative retinal neurotransmitter agents.

In the final adopted method for preparing the cultures, retinas were dissected from the eyes of embryonic day-19 rat embryos in a Ca^{++}-, Mg^{++}-free Dulbecco's phosphate buffer solution, exposed briefly to 0.05% trypsin, mechanically dissociated and then plated ($1\text{-}3\times10^{6}$ cells/plate) on collagen-coated 35mm plastic Petri dishes. The growth medium consisted of Nutrient Mixture F12 (HAM) and Dulbecco's Modified Eagle Medium (1:1) supplemented with 10% dialyzed fetal bovine serum. No antibiotics were used. The cultures were incubated without movement in a humidified air-CO_2 environment and fed daily (up to 8 weeks) with the growth medium. Intracellular recordings were made in balanced salt solution using conventional electrophysiological techniques.

Retinal neurons began emitting neurites soon after plating and the growth process stabilized by the end of the 2nd week. Beyond this time, cell death occurred but the majority of the remaining neurons increased in size(up to 20μm soma diameter). Intracellular filling with Lucifer Yellow revealed that many retinal neurons command processes with profuse arborizations and that several distinct types of neurons could be distinguished based on size and shape of the soma and branching pattern of the processes. Injection of depolarizing current pulses triggered action potentials, and spontaneous depolarizing or hyperpolarizing potentials or both were observed in most retinal neurons studied. Exogenously-applied GABA reversibly increased membrane conductance which was associated with a hyperpolarization and suppressed ongoing synaptic activity. Glutamate and acetylcholine in turn increased membrane conductance and produced depolarizing responses. One goal is to examine the interactions between retinal neurons which may utilize these substances as neurotransmitters.

In summary, mammalian retinal neurons are viable and synaptically-active under the present long-term culture conditions. This culture system should be useful for elucidating the physiological and pharmacological properties of retinal neurons.

249.1 DETECTABILITY OF THE RATE RESPONSE OF AUDITORY NERVE FIBERS NEAR MASKED THRESHOLD. E. D. Young, Dept. of Biomedical Engineering, Johns Hopkins University, Baltimore, Md. 21205

The psychophysical threshold for a tone masked by broadband noise is approximately a constant number of dB above the noise level. That is, as the noise level is increased, the masked threshold increases with approximately 1 dB of elevation per dB increase in noise level. The rate response of auditory nerve fibers behaves similarly in that rate versus level functions for best frequency tones are shifted, in the presence of noise, to higher sound levels (Costalupes et al., J. Neurophysiol. 51: 1326, 1984). However the shift, measured as displacement of the saturation point, increases with the noise level at only 0.6-0.8 dB/dB. The data suggest, however, that threshold behaves differently from saturation. This poster describes the behavior of rate response at masked threshold.

Rate versus level functions for tones in the presence of continuous background noise are expressed as detectability, defined as rate change (rate to tone plus noise minus rate to noise alone) divided by the standard deviation of the rate to the noise alone. This normalization gives an index of the ease of detection of the rate change produced by the tone. Responses are averaged across populations of fibers to allow small rate changes to be estimated reliably. As noise level increases, detectability decreases for two reasons. First, rate changes to tones decrease as fibers' responses to the background noise approach saturation rate. Second, the variability of the response rate to the noise alone increases as the rate to the noise increases. Three results are described. 1) The empirical variance of rate response is smaller than that predicted by the commonly-assumed Poisson process model for auditory nerve fiber spike trains. 2) Detectable rate changes are observed at noise levels up to at least 30 dB spectrum level. These rate changes are much larger in low and medium spontaneous rate fibers than in high spontaneous rate fibers, but are present in all groups of fibers. 3) As noise level increases, the threshold for rate change increases at 1 dB/dB. The thresholds for rate change correspond closely to cat's behavioral masked thresholds at the same noise levels.

Supported by grants from the National Institutes of Health.

249.2 RAPID ADAPTATION IN SINGLE AUDITORY NERVE FIBERS. L. A. Westerman and R. L. Smith. Institute for Sensory Research, Syracuse University, Syracuse , NY 13210.

Auditory nerve fibers respond to tone bursts with a high onset firing rate. The response then rapidly decreases (adapts). In gerbil, rapid adaptation appears to be well described by an exponential process with a time constant of several milliseconds (Westerman & Smith, Hearing Res., submitted). As sound intensity increases, the relative magnitude of the rapidly adapting component increases, while the time constant decreases, resulting in improved temporal resolution of the response onset. These effects are independent of the rise time of the stimulus for rise times of 5 msec or less. Thus the effects of transient spectral components at onset (frequency splatter and/or two-tone suppression) are not responsible for rapid adaptation. Similarly we have shown that rapid adaptation is not produced by refractory effects. At high intensities a given fiber fires with high probability at stimulus onset. The distribution of the interval between the onset spike and the next following spike reflects a short absolute refractory period during which the probability of another spike is zero. After this period the probability of a spike increases (relative refractory period). However, the conditional probability of the first occurrence of a spike reaches a maximum 1-3 msec after the onset spike, following which it rapidly decreases with a time constant similar to that of rapid adaptation. Furthermore, when a correction for refractory effects is applied to nerve fiber responses, using the model of Gaumond et al. (J. Acoust. Soc. Am. 74:1392-8, 1983), the resulting histograms still show a prominent rapidly adapting component. These results support the hypothesis that the rapidly adapting component of auditory nerve fiber responses reflects a corresponding decrease in the output of the synapse between the hair cell and auditory nerve fiber. Research supported by NIH and NSF.

249.3 Excitatory Amino Acid Pharmacology and Auditory Nerve Transmission in the Nucleus Magnocellularis of the Chicken. M. R. Martin, Lab. of Neuro-otolaryngology, NIH, Bethesda, MD 20205.

There is substantial evidence that an excitatory amino acid is released from the auditory nerve, activates NMDA type receptors in the cochlear nucleus of mammals and is terminated by an uptake process. In the present study a tissue slice preparation of the nucleus magnocellularis of the chicken, with the attached auditory nerve, is used to study the comparative pharmacology of this synapse.

A 400 μm transverse tissue slice of the 20-day chicken embryo brainstem was prepared and recordings were made in an interface-type recording chamber. Cells of the nucleus magnocellularis were antidromically and othrodromically stimulated using bipolar metal electrodes. Field potential recordings were made with low resistance glass electrodes filled with artificial cerebral spinal fluid. Evoked potentials were averaged and their amplitude and duration recorded. Once baseline conditions had been established drugs were added to the perfusion media in known concentrations.

Baclofen, a compound which blocks the release of excitatory amino acids, blocked orthodromically-evoked activity without affecting antidromically-evoked activity (ED_{50} = 4.9 μM). Kainate, N-methyl-D-aspartate and quisqualate, selective agonists for the three known excitatory amino acid receptors, induced alterations of antidromically-evoked activity. Threshold concentrations were 8 μM, 4 mM and 100 μM, respectively. 2,3-Cis Piperidine dicarboxylate (5 mM) reversibly blocked the actions of all three agonists. D-α-aminoadipate (5 mM) and glutamate diethylester (5 mM) reversibly blocked only the actions of N-methyl-D-aspartate and quisqualate, respectively. Of the three antagonists, only 2,3-cis-piperidine dicarboxylate affected orthodromically-evoked activity without affecting antidromically-evoked activity (ED_{50} = 2.0 mM). Dihydrokainate, a compound that blocks uptake of glutamate and aspartate, increased the duration of orthodromically-evoked activity (3-5 mM) without affecting antidromically-evoked activity.

The results suggest that, as in mammals, an excitatory amino acid is released from the chicken auditory nerve, it activates a kainate-type receptor on nucleus magnocellularis neurons (as opposed to the NMDA-type receptor found in mammals) and its action is terminated by an uptake process similar to those which exist for glutamate and aspartate.

249.4 ANALYSIS OF THE SURFACE ANATOMY AND SPATIAL TOPOGRAPHY OF THE COCHLEAR NUCLEI IN MAN WITH THE AID OF 3-D RECONSTRUCTION. L. Terr and B. Edgerton*, House Ear Institute, Los Angeles, CA 90057.

A program to develop and refine procedures for electrical stimulation of the cochlear nucleus (CN) to restore hearing has been undertaken by this institute. To determine the optimal surgical approach and electrode configuration, accurate physical models of the CN and adjacent structures of the brainstem were constructed. A synopsis of the technique used to derive the models follows: tissue blocks fixed in formalin were dissected from the area 20mm caudally and 20mm rostrally to the pontomedullary junction. Alignment holes were drilled in the frozen tissue blocks perpendicular to the caudorostral axis of the medulla oblongta. The tissue was sectioned in a cryostat; sections were 28μm thick and were stained with thyonin-luxol fast (Klüver and Barrera, 1953). Photographs of the sections were enlarged and the borders of the CN, adjacent parts of the vestibulocochlear nerve, tenia of the choroid plexus and brainstem were traced onto acrylic or polycarbonate plastic sheets. The sheets were cut, aligned, and assembled to form the models, which provide full medial, lateral, anterior, and posterior views of the CN and adjacent VIII nerve. The accurate localization of the cochlear nuclei boundaries on the brainstem surface was revealed. Quantitative 3-d topographical characteristics of the relief of the CN and a part of the VIII nerve adjacent to the pons were obtained. The attachment of the tenia of the choroid plexus to the CN surface was studied. A part of the CN was found hidden within the lateral recess and another part located outside of the recess. The portion of the CN outside of the recess is much smaller and forms a narrow strip adjacent to the posterior and ventral borders of the dorsal and ventral cochlear nucleus. A surgical approach to the area of the CN located within the recess was proposed and is being studied more extensively.

249.5 GOLGI-EM ANALYSIS OF SYNAPSES TO BUSHY CELLS IN THE POSTERIOR ANTEROVENTRAL COCHLEAR NUCLEUS (AVCN-P) OF THE CAT. E.-M. Ostapoff and D.K. Morest, Dept. of Anatomy, Univ. of Conn. Health Center, Farmington, CT 06032.

To understand the structural basis for signal processing requires knowledge of the synaptic inputs of the entire cell. In the AVCN-P, globular bushy cells have been shown to receive excitatory axosomatic endings characterized by large spherical (LS) vesicles which are thought to use acid amino acids as transmitters. However, the arrangement of the non-cochlear types of synaptic endings, especially on the dendrites, has not been so well studied and their functional properties remain to be elucidated.

Golgi-aldehyde impregnations of cochlear nuclei from 3 cats, 2-3.5 months old, were prepared by a gold substitution method. In each cat a well impregnated globular bushy cell was drawn in its entirety from 100 µm thick slices. The entire cell was sectioned serially at 3 µm and reconstructed at 8000 X with a computerized graphics system permitting 3-D rotations. For electron microscopy, thin sections were selected from each of five zones: axon hillock-initial segment, cell body, 1st-order, 2nd-order, and 3rd (or higher)-order dendrites. All endings from each sample were typed and their relative proportions determined.

The findings are that the LS endings are heavily concentrated on the cell body, axon hillock, and 1st-order dendrites. Terminals with pleomorphic or small spherical vesicles are preferentially distributed to higher-order dendrites. Endings with flattened vesicles are relatively evenly distributed on the cell but are more concentrated on the axon hillock.

LS endings provide synaptic security due to their heavy input to the soma and proximal processes. The post-synaptic effects of the other types of endings are uncertain. In other systems, flat vesicle endings make inhibitory synapses using GABA. If that is the case here, they could, by their arrangement, effectively suppress bushy cell output. Pleomorphic endings of the crossed olivo-cochlear bundle are thought to make inhibitory cholinergic synapses--in AVCN-P such endings could modulate dendritic potentials. Endings with small spherical vesicles have been implicated in excitatory synapses of granule cells using acidic amino acids as transmitters. The sources of all these endings (except for LS) will have to be determined before hypotheses concerning their function can be tested. Supported by PHS grant 5R01NS14347 and UConn Research Foundation.

249.6 **NEURONAL CIRCUITRY OF DORSAL COCHLEAR NUCLEUS: A COMPUTER MODEL.** **T.A.McMullen* and H.F.Voigt (spon: M.A.Casey). Dept. of Biomedical Engineering, Boston University, Boston, MA 02215.**

Single unit responses and cross-correlation analysis of type II and type IV units in the dorsal cochlear nucleus (DCN) of unanesthetized, decerebrate cats suggest that: 1) type II units inhibit type IV units and 2) type IV units share a source of spontaneously active input. We have attempted to test and explore these hypotheses of type II-type II and type IV-type IV functional interactions by digital computer simulation of neuronal circuitry in the DCN. The model includes a set of principal (P) cells and a set of interneuron (I) cells, both having the same best frequency. Both P and I cells receive excitatory input from primary and non-primary afferents, and I cells make inhibitory synapses on P cells. Details of the model are described elsewhere (Voigt and McMullen, Abstr. 7th midwinter meeting ARO, 67, 1984).

Results of the simulation demonstrate: 1) rate vs level functions of P cells and I cells are similar to those of type IV and type II units at best frequency. 2) Cross-correlograms of I-P cell pairs have an inhibitory trough similar to what is seen in the cross-correlograms of type II-type IV pairs in cat. 3) Cross-correlograms of P-P cell pairs often exhibit a central peak or mound similar to that observed in cross-correlograms of type IV-type IV pairs. We have found that the occurrence and strength of the central peak is related to the number of inhibitory inputs from the I cell population which are common to both P cells. The ratio of the peak to mean firing rate is an increasing function of the number of shared inhibitory inputs.

249.7 INTRACELLULAR RECORDINGS FROM BRAIN SLICES OF THE DORSAL COCHLEAR NUCLEUS OF THE MOUSE. J.A. Hirsch and D. Oertel, Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI 53706.

To learn how neuronal properties and interconnections in the cochlear nuclei shape responses to sounds, we made intracellular recordings in brain slices of the dorsal cochlear nuclei (DCN) of mice. We recorded synaptic responses to electrical stimulation of the auditory nerve and we studied the electrical properties of neurons. Slices, about 200µm thick, were made with a single, oblique cut tangential to the surface and included the stump of the auditory nerve and parts of the dorsal and ventral cochlear nuclei.

Electrical stimulation of the nerve evoked both excitatory (EPSP) and inhibitory (IPSP) synaptic potentials. The EPSPs were graded with stimulus strength and were almost always cut short by trains of IPSPs. The synaptic responses to stimulation of the nerve lasted as long as 90msec. Spontaneous IPSPs and action potentials were regularly recorded; we saw no evidence that spontaneous firing was triggered by EPSPs.

Intracellular injection of current showed that the electrical properties of cells in the DCN are complex. When depolarized by as little as 1mV from the resting potential (62±3mV, mean SD, n=17)many cells fired action potentials. These were either large, all-or-none and brief, or smaller and graded. The undershoots of action potentials comprised a fast component followed by a slower one whose duration varied. Though all cells fired large action potentials, different cells varied subtly and it is unclear whether we recorded from one or more anatomical classes of neurons.

Removal of extracellular Ca^{2+} and blockage of the voltage-sensitive Na^{+} channels with 1µM TTX allowed us to study the ionic basis of the electrical properties of cells in the DCN. In all cells that were tested (6/6), after extracellular Ca^{2+} was removed, cells fired in bursts and eventually remained depolarized until after Ca^{2+} was reintroduced, suggesting that normally Ca^{2+}-activated-K^{+} conductances help to maintain cells at their resting potentials. In TTX, the large action potentials disappeared, leaving the smaller graded ones. Subsequent removal of extracellular Ca^{2+} abolished all action potentials. These results indicate that in addition to Ca^{2+}-activated-K^{+} conductances, voltage-sensitive Na^{+} and Ca^{2+}conductances influence the electrical activity of cells in the DCN.

This work was supported by a grant from the National Institutes of Health, NS 17590.

249.8 INHIBITORY SYNAPTIC RESPONSES OF CELLS IN BRAIN SLICES OF THE VENTRAL COCHLEAR NUCLEUS. S.H. Wu* and D. Oertel, Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI 53706.

Synaptic responses of cells in the anteroventral cochlear nucleus (AVCN) to electrical stimulation of the auditory nerve have been recorded intracellularly in brain slice preparations (Oertel, J. Neurosci. 3:2043, 1983). In such preparations almost all cells respond to stimulation of the auditory nerve with excitatory synaptic potentials (EPSPs) which probably reflect direct excitation by auditory nerve fibers. In addition, most cells also respond with a later inhibitory synaptic potential (IPSP). Because IPSPs consistently have longer latencies than EPSPs and the latency and amplitude of IPSPs fluctuate in repeated measurements, they probably arise through a disynaptic pathway. Excitatory and inhibitory synaptic responses to electrical stimulation of the nerve are consistently recorded both in bushy and stellate cells, which can be distinguished by their intrinsic electrical properties (Wu and Oertel, J. Neurosci. 4, 1984).

To learn which neurotransmitters might be involved in mediating the IPSPs, we have tested the sensitivity of 69 cells in 34 slices to GABA and glycine and related blockers. IPSPs in both bushy and stellate cells were completely and reversibly blocked by 1µM strychnine (5/5 cells); they were not blocked by 0.1mM bicuculline (0/3 cells) or by 0.1mM picrotoxin (1/11 cells). GABA and glycine applied in the bath caused large, reversible concentration-dependent changes in input resistance. At low concentrations, no changes in input resistance could be measured; over a small (5-fold) concentration range the input resistance dropped steeply as a function of concentration; at higher concentrations the input resistance was so low as to be immeasurable. The lowest concentrations of GABA and glycine at which changes in input resistance could be detected, varied among cells from 0.1 to 10mM but was not correlated with cell type. Desensitization both to GABA and glycine was often observed. The amplitude of IPSPs was not altered by desensitization.

Several conclusions can be drawn from these experiments. Strychnine is a potent and specific blocker of IPSPs in the AVCN. Both bushy and stellate cells are sensitive to GABA and to glycine. Because desensitization to GABA and glycine did not affect the amplitude of IPSPs, however, it is possible that neither GABA or glycine mediate the IPSPs that we record in bushy and stellate cells.

This work was supported by a grant from the NIH, NS 17590.

249.9 **STIMULUS DEPENDENT NEURAL CORRELATIONS BETWEEN TYPE IV UNITS IN DORSAL COCHLEAR NUCLEUS.** H.F.Voigt, Dept. of Biomed. Eng., Boston Univ., Boston, MA 02215 and E.D.Young, Dept.of Biomed. Eng., Johns Hopkins Univ., Balto., MD 21205.

Neurons in the dorsal cochlear nucleus (DCN) of unanesthetized, decerebrate cats are classified physiologically on the basis of spontaneous activity (SPAC), and responses to 200ms best frequency (BF) tones. Type II units have little or no SPAC and give excitatory responses to BF tones of all levels. Type IV units are excited by low level 200ms BF tones, but are inhibited at higher levels. Results from a previous cross-correlation study are consistent with type II units providing inhibition to type IV units, and type IV units sharing a source(s) of spontaneously active input. Since type II units lack SPAC, they are not responsible for the correlations seen between type IV units in silence. Also, if type II units project to several type IV units, it may be possible to demonstrate correlated activity in type IV units uncorrelated in silence. We report here results obtained from 35 type IV - type IV pairs recorded simultaneously with two independently maneuvered microelectrodes. Of 31 pairs studied in silence, 6 had cross-correlograms (CCs) with a central mound indicating the presence of shared input; the others were featureless. CCs were obtained for 18 pairs under acoustic stimulation conditions as well as in silence. We used 60s, $\sqrt{BF_1 \times BF_2}$ tones at various levels above the units' thresholds at that frequency. Other frequencies were used when time permitted. In all cases (6) when the CC obtained in silence had a central mound, the CCs obtained under $\sqrt{BF_1 \times BF_2}$ stimulus conditions were also non-flat. Typically the size of the correlation was reduced for low SPLs, compared to the SPAC condition, and enhanced for moderate SPLs. At moderate levels, secondary features flanked the central mound. In 7 of 12 cases, the CCs were flat for both SPAC and acoustic stimulation conditions. In the remaining 5 of these 12 cases, however, the CCs obtained under acoustic stimulation exhibited central mounds of various sizes for certain frequency and level combinations. For one pair a frequency-level correlation map was constructed showing the tuning properties of the unit(s) responsible for the induced correlation. The derived tuning curve is V-shaped, lies within the inhibitory area of both type IV units, and may correspond to that of a type II unit or a low SPAC auditory-nerve fiber.

249.10 RELATIONSHIPS BETWEEN EXCITATORY/INHIBITORY RESPONSE TYPES AND SHORT-TONE RESPONSES IN COCHLEAR NUCLEUS OF DECEREBRATE CATS. W. P. Shofner and E. D. Young, Dept. of Biomedical Engineering, Johns Hopkins Univ., Baltimore, Md. 21205

Physiological classification of neurons in the cochlear nucleus (CN) has been based on discharge patterns in response to short tone bursts at best frequency (BF) or on the relative prominence of excitatory and inhibitory responses to tones and to noise. The first scheme has been studied in anesthetized animals, while the second has been studied mainly in unanesthetized, decerebrate animals. Because anesthesia can alter the properties of neurons, we have studied the characteristics of CN neurons in unanesthetized, decerebrate cats using both schemes.

Type I units give excitatory responses to tones and noise, and do not have inhibitory sidebands. Their discharge patterns are either primarylike or chopper. Some type I units have prepotentials. Most, but not all, of these give primarylike discharge patterns.

Type III units give excitatory responses to BF tones and noise, and have inhibitory sidebands. Most type III units give chopper discharge patterns. Such units are recorded throughout the CN. Some type III units in dorsal CN give pauser patterns, often with chopping at the onset peak.

Type I/III units give excitatory responses to tones and noise. They cannot be tested for inhibitory sidebands because they lack spontaneous activity. Most type I/III units give chopper discharge patterns. One group of type I/III choppers have monotonic BF rate versus level functions with sloping saturation.

Type II units are non-spontaneous and give excitatory responses to tones, but weak or no response to noise. These units give a variety of discharge patterns, and cannot be characterized as a group in terms of any particular discharge pattern. However, they have stereotypic rate versus level functions. The response latency for type II units is longer than any of the other types.

Type IV units give excitatory responses to noise and are characterized by their strongly non-monotonic rate versus level functions. They give inhibitory responses to tones over a wide range of frequencies, including BF. At levels of BF tones giving inhibition, type IV discharge patterns contain either on or on/off responses. The latencies of the on (off) responses are slightly shorter (longer) than type II units' response latencies.

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249.11 COCHLEA REMOVAL ELIMINATES PHYSIOLOGICAL ACTIVITY IN BRAIN STEM AUDITORY NUCLEI OF THE CHICKEN. D.E. Born* and E.W Rubel (SPON: D. Durham). Dept. of Otolaryngology, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

Rapid changes in the structure and metabolism of second order auditory neurons of nucleus magnocellularis (NM) occur following cochlea removal in young chickens. Since the ipsilateral cochlea provides the major excitatory input to NM neurons, it is of interest to determine the extent and time course of changes in physiological activity following peripheral manipulations. We investigated changes in spontaneous and acoustically evoked activity in NM and in its target, nucleus laminaris (NL), before and after removal of the cochlea.

Recordings were made from anesthetized 3 week old chickens maintained in a double walled IAC acoustic chamber. Metal electrodes (2-5 Mohms) were used in order to monitor activity from many neuronal elements. "Spike rates" were determined by setting a pulse height discriminator just above the non-physiological noise level of the recording system, where it remained for the entire experiment. Spontaneous spike rates were 500 - 1200 spikes/sec with this configuration. Activity in NM and NL was monitored before, during, and after each of the following manipulations: a) incision of the tympanic membrane; b) removal of the middle ear ossicle (columella); and c) removal of the basilar papilla (cochlea), leaving the eighth nerve ganglion cells intact. Each of these manipulations could be made without altering the recording field.

Following incision of the tympanic membrane or removal of the columella there was no change in NM or NL spontaneous spike rate. Removal of one cochlea eliminated spikes in the ipsilateral NM. NL receives binaural excitatory input. Removal of one cochlea caused a 10 - 20% decrease in spike rate. The effectiveness of stimulation of the remaining ear was unchanged. Removal of the second cochlea resulted in total cessation of NL activity within 30 seconds. There was no recovery of activity for up to six hours. We conclude that all excitatory physiological activity in the cochlear nerve and NM neurons is of receptor origin. Rapid structural and metabolic changes following cochlea removal could be due to the immediate and complete cessation of physiological activity.

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249.12 GAD-LIKE IMMUNOREACTIVITY IN THE COCHLEAR NUCLEI AND SUPERIOR OLIVARY COMPLEX. J. K. Moore[1] and R. Y. Moore[2]. Departments of Anatomical Sciences[1], Neurology[2], and Neurobiology[2], SUNY at Stony Brook, Stony Brook, NY 11794

Glutamic acid decarboxylase-like immunoreactive (GADLI) neuronal somata and terminals were demonstrated in the lower brainstem auditory nuclei of the rat and guinea pig using the PAP method with an antibody to rat brain GAD (kindly supplied by I. Kopin and W. Oertel).

Cochlear nuclei. GADLI cell bodies are present only in the outer layers of the dorsal nucleus: these are small (15 μm) neurons with scant cytoplasm. GADLI terminals are distributed through the entire dorsal nucleus but more densely in its outer molecular and granular layers than in the central region. In the ventral nucleus, GADLI terminals are numerous in all regions except the nerveroot and bundles of eighth nerve axons, which show no immunoreactivity. In some areas, particularly along the medial and lateral margin of the nucleus, GADLI terminals form dense pericellular arrays which outline the somata of smaller neurons and presumably represent a dense axosomatic innervation. Small fascicles of GADLI vericose axons are observed adjacent to the nuclei in the acoustic stria and vestibular nerve.

Superior olivary complex. The lateral olivary nucleus contains a population of GADLI neurons whose size, shape, and distribution suggest that they may represent a subset of the principal neurons of the nucleus. In addition, sparse strands of terminals radiate from the hilus of the nucleus. In the medial olivary nucleus, no GADLI neurons are present but terminals are distributed over the somata and horizontal dendrites of its neurons. In the nucleus of the trapezoid body, GADLI terminals surround the oval somata of its cells and continue over the proximal dendrites. The periolivary nuclei generally contain scattered immunoreactive terminals and lightly stained round or fusiform cells. However, in the ventral periolivary region there is an extremely dense band of GADLI terminals and a group of intensely reactive perikarya.

These observations indicate that GADLI neurons are rather restricted in their distribution, but that GADLI terminals are so ubiquitous that they can potentially influence the processing of auditory information in all subdivisions of the primary and secondary brainstem auditory nuclei.

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249.13 RESPONSE PROPERTIES OF TRAPEZOID BODY FIBERS. G.A. Spirou*, W.E. Brownell, and M. Zidanic*. Dept. of Neuroscience, Univ. of Florida, Gainesville, FL, 32610.

Single units have been recorded from the midline trapezoid body of adult cats either decerebrate or under barbiturate anesthesia using both glass and Pt-Ir microelectrodes. In addition, the spatial distribution of click evoked field potentials have been measured within the trapezoid body.

Thirty-one units have been sampled, 18 from barbiturate cats and 13 from decerebrate cats. The average spontaneous rate was 11 ± 14 in barbiturate cats and 38 ± 23 in decerebrate cats. PSTH's were generated at 30 and 50 dB above threshold at the characteristic frequency, and do not differ greatly between the groups. The predominant PST type is primary-like, although some show a slow decline to low rates throughout the stimulus (50 msec duration). Several units have a large onset response followed by low rate sustained activity. Units in decerebrate cats were more difficult to hold for long periods; only 3 units were adequately investigated for receptive field properties. One of these units, localized to the population of large diameter fibers, showed weak lower inhibitory sidebands comparable to those recorded in barbiturate preparations (Brownell, Br. Res. 94: 413, 1975). This unit had a low spontaneous rate of 10s/s and a primary-like PSTH. Two medium spontaneous rate units had no inhibitory sidebands. Phase locking to low frequencies has been measured in detail in 2 units, and from this information delay times were calculated to be 3.95 and 3.75 msec. These values correlate well with tone evoked spike latencies, using a rise time of 2.5 msec.

Click evoked field ptoentials recorded at the midline consist of 5 prominent negative peaks. This pattern occurs in both barbiturate and decerebrate cats, using either monaural or binaural activation. As the electrode advances from the ventral surface over the pyramids N3 at 2.0 msec increases in amplitude then decreases. While N3 is decreasing, N4 at 2.5 msec increases then decreases at the most dorsal locations. Single unit click latencies generally fall between 2.0 and 2.5 msec for high frequency units.

The increased spontaneous rate of units from decerebrate cats should permit demonstration of inhibitory processes in the ventral cochlear nucleus that may not be revealed under barbiturate anesthesia. Further experiments are aimed toward interpreting evoked potential waveforms in terms of trapezoid body and superior olive neural generators.

249.14 MORPHOLOGICAL CHARACTERISTICS OF THREE NEURONAL TYPES IN THE CAT LATERAL SUPERIOR OLIVARY NUCLEUS. R.H. Helfert and I.R. Schwartz. Departments of Anatomy and Head and Neck Surgery, UCLA School of Medicine, Los Angeles, CA, 90024.

Three distinct neuronal types: principal (PR), multipolar (MU), and marginal (MA); can be observed in Golgi impregnated material from the lateral superior olivary nucleus (LSO) of kittens and adult cats. Further evidence of multiple neuronal classes within the LSO is provided by differences in the Nissl stains of perikaryal cytoplasm, and the electron microscopic observations of at least two discrete patterns of synaptic terminal distribution on neuronal perikarya.

The most abundant cell type, recognized by Ramon y Cajal, is uniplanar and multipolar, oriented rostrocaudally in a plane perpendicular to the transverse curves of the LSO (Scheibel and Scheibel, Exp. Neurol. 43:339,1974). In transverse sections, the somas of these "discoid" PR cells appear fusiform and bipolar, 16-35μm long, 6-15μm wide, varying in shape from a narrow spindle to a broad oval. Approximately 85% of the surface of PR cells in the middle limb is apposed by synaptic terminals, primarily those containing flat vesicles (Cant, Neurosci. Abst. 9:766, 1983). We see a similar arrangement on the PR cells in the lateral and medial limbs.

The less frequently observed MU cells are distinguished in transverse Golgi sections by their multipolar shape, in clear contrast to the fusiform shape of the PR cells. In the same plane, Nissl stained MU cells appear round or polygonal. Some of the largest perikarya in the LSO matrix, MU as well as the largest PR cells, have a diffuse Nissl pattern. Electron micrographs show a pattern of synaptic input to the MU cell similar to that observed in the PR cells.

The MA neuron is recognized by its shape and its characteristic location at the contours of the LSO, immediately beneath the myelinated fibers encircling the nucleus. In the Nissl stained transverse sections, MA cells are seen as bipolar, oriented perpendicularly to the PR cells and found throughout the borders of the LSO. An MA cell axon was observed forming a terminal plexus within the LSO.

Throughout the matrix of the LSO, a few cells are observed whose perikaryal surface is largely covered by thin glial sheets; with only an occasional, usually small, synaptic terminal. It is not yet known which neuronal type has this pattern of somal contacts. Studies are continuing to further describe and correlate the specific characteristics of the neuronal and dendritic shapes with the innervation patterns of the multiple classes of LSO neurons.

Supported by NS 09823, NS 14503, and NSRA NS 07059.

249.15 INTERAURAL TIME SENSITIVITY IN THE MEDIAL SUPERIOR OLIVE OF THE CAT: COMPARISONS WITH THE INFERIOR COLLICULUS. J.C.K. Chan and T.C.T. Yin. Dept. of Neurophysiology, Univ. of Wisconsin Medical School, Madison, WI 53706.

The superior olivary complex (SOC) is the primary site of binaural convergence in the auditory system. We studied single neurons in the SOC of barbiturate-anesthetized cats by recording extracellularly while stimulating dichotically with interaurally delayed tones and noise as well as binaural beats. This report focuses on low-frequency cells that were located in or around the medial superior olive (MSO), as indicated by the locations of lesions made at the time of recording. These cells provide a major input to the low-frequency cells of the central nucleus of the inferior colliculus (ICC), which has been the subject of our previous studies.

As in the ICC, most cells in the MSO are sensitive to interaural phase and a large majority (80%) of these showed a characteristic delay (CD) as determined by the objective criteria of Yin and Kuwada (1983). Unlike the ICC, however, cells in the MSO usually have their CDs at the point of maximal discharge. The interaural phase at which the discharge rate was maximal could be predicted from the monaural responses, which were phase-locked to stimulation of either ear for about two-thirds of the cells. This supports the coincidence model of Jeffress (1948) and agrees with earlier studies of the MSO. The values of the CD generally fell within the physiological range of interaural time delays (ITDs) for the cat (400 μsec) and showed a strong bias towards signals with delays to the ipsilateral stimulus. When tonal responses at different frequencies were summed for each cell to form a composite response curve, the distribution of the peaks of these curves was similar to the distribution of CDs. These cells also showed ITD sensitivity to broad band noise stimuli and these responses were predictable from their tonal composite response curves.

We conclude that the responses of most cells in the MSO are in agreement with a coincidence model of binaural interaction. The tendency for binaural cells at all levels of the auditory system above the SOC to respond preferentially to sound sources in the contralateral sound field is set up initially for low frequencies in the MSO. Furthermore, there must be additional binaural processing at the level of the inferior colliculus.

Supported by NIH grants EY02606 and NS12732.

249.16 AUDITORY BRAINSTEM CONNECTIONS TO AND FROM THE CENTRAL NUCLEUS OF THE TORUS SEMICIRCULARIS IN THE RED-EARED TURTLE, CHRYSEMYS SCRIPTA ELEGANS. R.H. Browner, D. Marbey and D.M. Pierz. Dept. of Anatomy, New York Medical College, Valhalla, N.Y. 10595.

TS is located deep to the optic tectum and in the floor of the optic tectal ventricle (Browner et al., J. Morph. 169:207-223, '81). The turtle was anesthetized with Brevital Sodium (0.70%) and then maintained with oxygen (250 cc/min), halothane (0.5-2%), and nitrous oxide (100 cc/min) on a stereotaxic apparatus. The muscles were removed over the dorsolateral skull and the bone removed. The meninges were cauterized, the optic tectum cut away and the tectal ventricle exposed. The floor of the tectal ventricle, formed by the CN and laminar nuclei, was penetrated by a glass micropipette filled with 25% horseradish peroxidase (HRP, Sigma type VI). The HRP was injected into the CN by pressure or iontophoresis for 10 to 15 mins. Following the injection gelfoam was placed over the opening in the tectal ventricle, the skull sealed with dental cement and the skin wound clipped closed. Seven animals survived from 5 to 7 days and were perfused and reacted for HRP with the TMB method (Browner, Hearing Res., 12:139-143, '83). Afferent and efferent fibers from the CN course through the lateral lemniscus (LL). This tract coursed ventrally and caudally to the rostral end of the medulla. Here the fibers separated into at least 3 separate tracts. The ventral tract followed the basal surface of the brainstem and crossed the midline to the contralateral side. In its pathway it intercepted the ipsilateral and contralateral SO where there were filled neurons. The ventral route continued caudally and turned dorsally to reach the contralateral acoustic tubercle (AT); as well as a small input to the ipsilateral AT. The intermediate pathway fanned out medially across the brainstem and entered the contralateral (AT). The dorsalmost group coursed ventral to the 4th ventricle and the medial longitudinal fasciculus to reach the contralateral (AT). The CN received the majority of its input from the contralateral auditory brainstem nuclei. Ipsilaterally the afferents came from the superior olive (SO), the nuclei magnocellularis (NM) and laminaris (NL), contralaterally from the SO, NM, NL and to the nucleus angularis (NA). In addition there was an efferent projection from the CN to the contralateral NM and NL. A few cells were labelled in the ipsilateral nucleus of the lateral lemniscus.

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250.1 MORPHOLOGY OF PHYSIOLOGICALLY CHARACTERIZED CELLS IN RAT MEDULLARY DORSAL HORN (MDH). W.E. Renehan*, M.F. Jacquin, R.D. Mooney & R.W. Rhoades (SPON: W. Graham). Dept. of Anatomy, Univ. of Med. & Dent. of N.J.- School of Osteopathic Med. & Rutgers Med. School, Piscataway, N.J. 08854

We (Jacquin et.al., Br. Res.,'84) have previously shown that vibrissae (VIB) and guard hair (GH) primary afferents each have distinct arborization patterns in the lamina III-IV region of the MDH. In the present study, standard intracellular-HRP techniques were used to determine whether such a distinction extended to MDH cells. Thus far, we have recovered 7 cells which responded only to VIB deflection, 7 which were sensitive only to GH stimulation and 6 which could be activated by both VIB and GH. All of these cells had no spontaneous activity, responded in a rapidly adapting fashion to gentle deflection of the appropriate hair(s) and were driven by trigeminal ganglion shocks with latencies of 1.0-2.1 ms. The morphologies of cells within a given physiologically defined class were quite diverse. The somata of all VIB-only cells were located in the deep ½ of layer III-IV or lamina V, and their dendrites were restricted to all or some portion of these laminae. The dendritic arbors of VIB-sensitive neurons did not appear to differ as a function of the number of VIB (1-21) which could activate the cell. Two of these cells had their axon enter the trigeminal spinal tract. Only one of these neurons could be antidromically activated from the contralateral thalamus, and its axon gave off collaterals in the deep reticular core prior to decussation.

The somata of neurons sensitive only to GH stimulation were all located in the lamina III-IV region and, within the limits of our small sample, tended to be more dorsal than those of VIB-only cells. Dendrites of these cells were located in laminae II-IV. None of these neurons were antidromically activated by thalamic stimulation.

Neurons sensitive to both VIB and GH stimulation had somata in layers III-V. The dendritic arbors of these cells were quite varied, but some extended to lamina I. Overall, they encompassed layers I-V. One of these cells had an axon which innervated laminae I and II almost exclusively. Another was antidromically activated by thalamic shocks and its axon also collateralized in the reticular formation prior to decussation. While our sample is quite small, the data do suggest that MDH neurons with GH input may be distinguished from those with only VIB input by a tendency to have more dorsally located somata, as well as axons and dendrites which often extend into layers I & II. Support: DE06528, EY04170,EY03546, March of Dimes, UMDNJ Foundation (RWR), NRSA NS07444 (WER).

250.2 MORPHOLOGY OF PHYSIOLOGICALLY CHARACTERIZED PRIMARY AFFERENTS IN RAT SPINAL TRIGEMINAL SUBNUCLEUS INTERPOLARIS (SpVi). M.F. Jacquin, W.E. Renehan*, R.D. Mooney & R.W. Rhoades. Dept. of Anatomy, Univ. of Med. & Dent. of N.J. - School of Osteopathic Med. & Rutgers Med. Sch., Piscataway,N.J. 08854

We have previously shown that vibrissae-sensitive primary afferents (PA's), regardless of their functional characteristics, arborize in a restricted portion of the lamina III-IV region in the medullary dorsal horn (MDH); while guard hair PA's collateralize more diffusely in layers III-V (Jacquin et al., Br. Res.,'84). In the present study, we have analyzed the collaterals of these same fibers in SpVi, a non-laminated V subnucleus. Thus far, we have recovered 26 identified HRP-labeled PA's which gave off collaterals in both the MDH and SpVi. Nineteen were sensitive only to stimulation of 1 long sinus hair (slowly adapting type I:N=6, type II:N=5; rapidly adapting:N=6; vibration-biased:N=1; velocity-biased:N=1); 6 responded only to guard hair stimulation. As in the MDH, functionally distinct vibrissae-sensitive PA's were morphologically indistinguishable. Each provided a largely continuous series of radially oriented collaterals that gave rise to densely packed and highly circumscribed terminal arbors.

Contrary to a previous report (Hayashi, Br. Res.,'82) using identical methods, low threshold cutaneous axons arborized in a manner consistent with other histochemical, transganglionic tracing and electrophysiological studies. PA's with rostral facial receptive fields (RF's) terminated medially, while those with caudal RF's arborized laterally. PA's with dorsal RF's innervated ventral SpVi and those with ventral RF's terminated dorsally. Axon arbors in SpVi did not appear to follow an "onion leaf"-like rostrocaudal topography. Thus, unlike the case in the MDH, the entire ipsilateral face was represented at each rostrocaudal level. The terminal arbors of low threshold PA's were also, as a whole, considerably smaller in SpVi than in the MDH. This may be due to the above-described differences in topographic organization.

One additional PA responded only to a noxious pinch deep to the vibrissae pad and its latency to V ganglion shocks was much longer than that of the above-described PA's. While arborizing extensively in laminae I,II, and V in the MDH, this fiber did not innervate SpVi, but did give off 1 collateral into a gelatinous interstitial pocket in the V spinal tract lateral to SpVi. Studies in progress will determine the extent to which vibrissae, guard hair, and nociceptive-biased PA's are qualitatively distinguishable in SpVi, as they are in the MDH. Supported by DE06528, EY04170, EY03546, the March of Dimes, the UMDNJ Foundation (RWR), NRSA NS07444 (WR)

250.3 CYTOARCHITECTURE, HISTOCHEMICAL AND GOLGI STUDIES OF THE CELLS IN THE REGION OF THE BRACHIUM CONJUNCTIVUM OF THE GUINEA PIG. R.R. Pindzola and R.E. Foster. Neurotoxicology Branch, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010

The morphological characteristics of neurons in the caudal parabrachial area (PBA; posterior to the inferior colliculus) were studied using Golgi-Cox and Nissl stained preparations of young guinea pig brains. Acetylcholinesterase (AChE) distribution was studied in the same area by use of the enzyme histochemical stain of Koelle. The guinea pig PBA is partitioned by the brachium conjunctivum (BC) into medial and lateral divisions which, in turn, can be subdivided by cytoarchitectonics. In contrast, neither myeloarchitectonics nor AChE enzyme histochemistry provide criteria for further subdivision of the two PBA divisions. The distribution of AChE in the two divisions is fairly uniform with the content being much less than the adjacent motor V nucleus. Cytoarchitectonic analysis of the medial and lateral PBA reveals the following: 1) the lateral division consists of peribrachial, intermediate, external and ventral cell groups and 2) the medial division consists of peribrachial and medial cell groups. There are also scattered cells in the BC proper which we refer to as the interstitial cell group. In Nissl preparations the interstitial BC neurons are oval, approximately 13.5-21μ m in size. The neurons nearest to the BC (medial and lateral peribrachial) are fusiform in the sagittal plane with an average size of 18 x 7μ m. The neurons of the intermediate, external and ventral lateral PBA are multipolar or slightly fusiform cells. Neurons in the medial PBA vary, the cells proximal to the brachium being more fusiform and than the cells more distally located. Based on Golgi-Cox impregnations of neurons in the different PBA cell groups, it appears that the interstitial BC neurons and the immediately adjacent peribrachial cells are relatively simple with the several primary dendrites having few branches devoid of appendages. In contrast, neurons in the PBA cell groups distal to the BC are more complex with greater dendritic branching and larger numbers of dendritic appendages/-spines. Neurons in the lateral intermediate cell group appear the most complex. While the cell bodies of the fusiform PBA neurons are oriented in the sagittal plane in Nissl stained preparations, their dendrites seem not to have a preferred orientation. Thus, from our architectonic analyses there are at least seven subdivisions of the PBA in the guinea pig.

250.4 INTRACELLULAR RECORDINGS FROM MITRAL/TUFTED CELLS IN THE OLFACTORY BULB OF THE TIGER SALAMANDER. K.A. Hamilton and J.S. Kauer. Depts. of Neurosurgery; Anatomy and Cell Biology, Tufts- New England Medical Center, Boston, MA 02111.

In order to study integrative mechanisms in the olfactory bulb, we are recording the intracellular responses of mitral/tufted cells to electrical stimulation and to different odorants at a range of concentrations. The cells subsequently are stained for morphological examination using a horseradish peroxidase (HRP) technique.

The mitral/tufted cells spike actively, usually at rates of between 1 and 5 impulses/sec. Electrical stimulation of the medial olfactory tract or olfactory nerve, or application of an odorant to the olfactory mucosa, usually results in sustained hyperpolarization and suppression of spiking for up to 7 sec. The suppression is longer in response to a suprathreshold electrical stimulus than in response to the threshold stimulus that will elicit a single spike preceding the suppression. In some cells the initial portion of the response to orthodromic stimulation includes a brief depolarization and a burst of spikes, the number of which increases with stimulus intensity under certain stimulus conditions.

Numerous branches extend from the stained cell bodies into the plexiform layer, and at least in several of the cells dendrites branch in two or more glomeruli. The staining characteristics indicate the recording/staining microelectrode containing the HRP sometimes is positioned in the plexiform layer at the level of the larger dendrites, rather than at the level of the cell body. The characteristics of the recordings often support the idea that they are obtained from dendrites or that dendritic spikes propagate to the recording site.

The interactions of the mitral/tufted cells with other types of cells forming the layers of the bulb are being studied using combined electrical and odorant stimulations.

Supported by NS-20003 (JSK).

250.5 VOLTAGE-SENSITIVE DYE RECORDING FROM THE OLFACTORY SYSTEM OF THE TIGER SALAMANDER. J.S. Kauer, D. Senseman* and L.B. Cohen. Depts. Neurosurgery, Anatomy and Cell Biology, Tufts-New England Medical Center, Boston, MA 02111; Div. Life Sciences, Univ. Texas, San Antonio, TX 78285; Dept. Physiol., Yale Univ. Sch. Medicine, New Haven, CT 06510.

A voltage-sensitive dye has been used to monitor neuronal activity in the intact salamander olfactory bulb after odor stimulation of the mucosa. Certain dyes, when incorporated into neuronal membranes, change their fluorescent properties with voltage change across the membrane. By recording the optical signals generated by the dyes, one can measure voltage changes in neuronal tissue without the use of invasive electrode penetrations. By using an array of optical recording devices, signals from a number of sites can be measured simultaneously with a time resolution of 0.7 msec (review- Cohen and Salzberg, 1978, Rev. Physiol. Biochem. Pharm. 83:35).

In the present study the styryl dye RH414 has been used to observe activity in the olfactory bulb after stimulation of the nose. A square array of 124 detectors was centered over the bulb to record fluorescence changes with both electrical and controlled odor stimulation. In this way sets of large signals were recorded which were aligned parallel to the bulbar layers. Unlike bulbar layers of the mammal, the cell layers in the salamander are stacked in a planar configuration and extend to the surface of the bulb so that the fluorescence signals can be correlated directly with the bulbar strata. These studies allow observation of the spatial distribution of rapid neuronal events and are being compared with the spatial distibution of activity we have observed using the 2-deoxyglucose method.

Supported by USPHS grants NS-20003 (JSK) and NS-80437 (LBC).

250.6 SOMATOTOPIC ORGANIZATION AND BIMODAL REPRESENTATION IN THE ELECTRORECEPTIVE MIDBRAIN OF THE ELASMOBRANCH, PLATYRHINOIDIS TRISERIATA. J. Schweitzer. Neurobiology Unit, Scripps Inst. of Oceanog., Univ. of Calif., San Diego, La Jolla, CA 92093

Electrosensory receptive fields in the thornback ray are somatotopically organized along a rostro-caudal extent of the neuraxis previously divided into 2 distinct electrosensory nuclei on the basis of anatomical (Bodznick, D.A., Northcutt, R.G. Brain Res., in press) and physiological (Schweitzer, J. J. Comp. Physiol., 153:331-341, 1983) evidence. The finding of a single, continuous, unfractured map extending from the rostral tip of the anterior nucleus (AN) to the caudal border of the lateral mesencephalic nucleus (LMN) now suggests that LMN and AN are in fact a single mesencephalic structure.

Minimum receptive fields (RFs) of single electrosensory units or multiunit clusters, first isolated in response to a uniform electric field, were determined with a just-suprathreshold 500 ms square wave electric stimulus produced between a remote stationary reference and a roving chlorided silver-ball monopole attached to a flexible manipulator arm. Receptive fields of the anterior, mid-, and posterior body were recorded in the rostral, mid-, and caudal regions of dorsal AN-LMN, respectively. All receptive fields restricted to the ventral surface (78%) are contralateral and are rarely larger than 1 cm^2. The remaining units have disjunct receptive fields on both the dorsal and ventral surfaces; the position of one is not predictive of the other and the dorsal field can be either ipsi- or contralateral. Somatotopy has not been demonstrated in the ventral aspect of this nucleus or from the dorsal surface of the body.

Nearly all electrosensory units are bimodal, responding vigorously to the gentle touch of a camel-hair brush. Ascending spinal input to the shark midbrain previously demonstrated by degeneration (Ebbesson, S.O.E., Hodde, K.C. Cell Tiss. Res., 216:313-331, 1981) provides an anatomical basis for this mechanosensitivity. Mechanosensory RFs are generally larger than but invariably overlap with electrosensory RFs, demonstrating that the somatotopic maps of the 2 modalities are nearly coincident. This evidence suggests that, as in other vertebrates, the midbrain in elasmobranchs is an important center for multimodal sensory integration.

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EEG AND ERP

251.1 HUMAN MEDIAL TEMPORAL LOBE POTENTIALS EVOKED IN MEMORY AND LANGUAGE TASKS. M.E. Smith, J.M. Stapleton and E. Halgren, Lab. for Cognitive Neurophysiology, Brain Res. Inst., Univ. Calif. at Los Angeles, 90024.

We have previously reported that event-related potentials (ERPs) can be recorded from the human medial temporal lobe (MTL) during recent memory tasks (Soc Neurosci 9:647). Further studies have clarified the component structure of these ERPs, their psychological correlates, and their relationship to scalp recorded potentials. Recordings were obtained from the amygdala, hippocampus, and parahippocampal gyrus, and from the calvarial electrodes placed at 10-20 coordinates, in 13 patients being evaluated for surgical treatment of epilepsy. Evoking tasks included detection of repeated words in a recent memory recognition test, discriminating words from non-words, cued-report naming of pictures, and mental counting of "rare" high pitched tones in a train of low pitched tones.

Tasks requiring primary memory and semantic analysis for their performance (lexical decision and naming) were observed to elicit a component peaking around 400ms post-stimulus onset. This component was negative at the scalp and usually negative in the MTL. It was similar in latency, morphology, and task correlates to the N4 component of the scalp recorded ERP reported by others to be elicited in similar tasks (Bentin et al. Soc Neurosci 9:1199,1983; Stuss et al. EEG Clin Neurophys 56:133-146, 1983). This same component was also reliably elicited during verbal tasks requiring secondary memory for their performance, where it decreases in amplitude to repeated items during memory testing. The N4 component reverses polarity over short distances in the hippocampus and parahippocampal gyrus, and attenuates in amplitude at more lateral recording sites. A second endogenous component occurs after the N4, and its depth distribution was identical to the P3 component of the MTL response to rare stimuli and was distinct from the distribution of the N4 component. This positive component increases in amplitude to repeated words. We conclude that the MTL N4 component observed in these tasks is probably of local origin, but generated by different synapses than those involved with the MTL P3 response. The decrease in N4 amplitude associated with activation in Primary Memory, previously demonstrated by other authors, has been extended here to repeated words in Secondary Memory. We gratefully acknowledge the collaboration of P. Crandall, J. Engel, Jr., W. Sutherling, L. Cahan, M. Nuwer, and the staff of the Clinical Neurophysiology Program at UCLA. Supported by USPHS Grant NS18741.

251.2 DEPTH AND SURFACE COMPONENTS OF POTENTIALS EVOKED IN SIMPLE COGNITIVE TASKS. J. M. Stapleton and E. Halgren. Brain Research Institute, UCLA and VA Southwest Regional Epilepsy Center, Los Angeles, CA.

We have been studying potentials evoked differentially in several simple cognitive tasks, including AA- counting rare high tones in a series of tones (1400 ms ISI), AI- ignoring the same stimuli, AD- same as AA but with occasional nontarget strange sounds, OM- counting omissions of tones in a series (600 ms ISI), CO- counting all tones (random 3 to 9 sec ISI), VO- similar to AA in the visual modality. Data have been collected for some or all tasks for 24 patients undergoing diagnostic depth electrode evaluation for epilepsy, and 15 normal subjects (22 scalp sites, 10-20 system). In the depth, there are multiple components with both time and task relationships to the surface N1-P2-N2-P3-SW. These components may be revealed by detailed visual inspection of the data, by quantitative analysis using Principal Components Analysis, or by measurement of peak amplitudes in latency windows defined by surface components. There are differences among them in both depth and scalp topography.

A distinction may be drawn between two varieties of endogenous limbic potentials (ELPs). The large, polarity-reversing potentials (ELPb) previously found in Medial Temporal Lobe (MTL) in the latency range of the surface P3 and SW have been replicated in this series of patients. They are often very large and negative in the anterior hippocampus, large and positive in other MTL sites, and fall rapidly in amplitude in more superficial sites. An additional negative-positive component (ELPa) is evident in many depth sites just before the surface N2/P3. Although it also may reverse polarity over short distances within the MTL, it declines relatively little in amplitude as the surface is approached. Both ELPa and ELPb are largest to rare, attended stimuli, but ELPa is relatively enhanced to weird, nontarget sounds in AD. Both are also evoked by CO, although sometimes at shorter latency than in AA.

Depth recordings appear to be useful in separating evoked potential components that are difficult to distinguish in scalp recordings because they overlap in latency, topography, and/or task correlates.

Supported by NS18741 and by the Veterans Admin. We acknowledge the collaboration of P. Crandall and J. Engel, Jr.

251.3 IS N400 SPECIFICALLY RELATED TO THE PROCESSINF OF SEMANTIC MISMATCH ? M. Besson*, F. Macar* and J. Pynte* (SPON : J. Requin). Dept. Psychobiol. exper., Inst. Neurophysiol. Psychophysiol., Marseille, France.

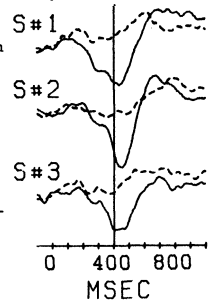
Kutas and Hillyard (1980, 1982) showed the existence of a component peaking 400 msec after the presentation of an incongruous word within a given sentence context. The aim of the present study is to verify whether the occurrence of N400 depends on the overlearned nature of the mechanisms involved in tasks such as reading or whether N400 also appears when an arbitrary rule learned during the experiment is violated. Visual and auditory modalities were tested. Four experimental conditions were used. The two first involved overlearned relations between the probe stimuli and their context. In the other two, probe stimuli were linked to their context by a logical and arbitrary rule. The conditions involved: 1) Words in the context of sentences visually presented. 2) Musical notes of well-known French melodies auditory presented. 3) Geometric figures ordered in increasing or decreasing size, visually presented. 4) Scale-notes, auditory presented. For 25% of the trials in each condition the last stimulus was incongruous. The start of each trial was signaled by the apparition of a line of X's, at the center of a screen, during 500 msec and followed 1500 msec later by the sequential presentation of seven stimuli lasting 700 msec each, at the rate of one stimulus per sec.

EEG recordings from Fz, Cz, Pz, W2 and W1 confirm that N400 only occurs when there exists a semantic mismatch between words. In the other situations, incongruous stimuli produce P300, which are largest in the melodies. Clear-cut differences also appear right from the N1 component, larger in the auditory conditions, and for incongruous compared to congruous stimuli in all conditions. Changes in the amplitude and duration of these three components can be interpreted as directly related to the complexity of the information being processed.

Kutas, M., Hillyard, S.A., 1980. Science, 207, 203-205.
Kutas, M., Hillyard, S.A., 1982. Neuropsychologia, 20, 579-590.

251.4 INTRACRANIALLY RECORDED EVENT-RELATED POTENTIALS DURING SENTENCE PROCESSING. G. McCarthy* and C.C. Wood. VA Medical Center, West Haven, CT 06516 and Yale U., New Haven, CT 06520.

Kutas and Hillyard (Science, 1980, 207:203-205; Nature, 1984, 307:161-163) described a negative-going deflection ("N400") in scalp ERPs that appears to be sensitive to the amount of semantic priming or expectancy for a stimulus word created by its sentence context. Here we describe the morphology and intracranial distribution of ERPs recorded in chronically implanted epileptic patients under the same conditions which elicit N400 in scalp recordings. Recordings were obtained from 3-6 depth probes (18 contacts per probe) in each of 11 patients undergoing chronic depth EEG monitoring as part of an extensive evaluation for possible epilepsy surgery. Six- to twelve-word sentences were presented one word at a time on a CRT screen (exposure duration: 200 msec; interval between word onsets: 500 msec; interval between sentences: 3-4 sec). Final words were semantically correct in half the sentences, and anomalous in the other half. As in the initial Kutas and Hillyard studies, subjects were instructed to read the sentences silently for understanding and were informed that some of them might seem odd. ERPs were recorded simultaneously from 50-64 intracranial electrode sites (4 or 6 msec/point), beginning 100 msec before the onset of the last word in each sentence. The largest and most consistent difference between ERPs for semantically anomalous and correct sentences (solid and dotted lines in figure) was a prominent negative deflection peaking between 400 and 500 msec. This activity was focally distributed at electrode sites in the vicinity of the amygdala and anterior temporal pole (ERPs from this region are shown in the figure for three different subjects; cal: 20μV), and was much smaller both posteriorly near the hippocampus and dorsofrontally in orbital-frontal cortex. Thus, its distribution was distinct from that of temporal lobe potentials coincident with scalp P300 which are maximal in the vicinity of the hippocampus (McCarthy et al., Soc. Neurosci. Abs., 1982, 8:976).



(Supported by the Veterans Administration and NIMH Grant MH-05286).

251.5 ERP AND P300 ACTIVITY IN PATIENTS FOLLOWING UNILATERAL TEMPORAL LOBECTOMY. R. Johnson, Jr.* and P. Fedio* (SPON: J. Rohrbaugh). NIH/NINCDS/Clinical Neurosciences Branch, Building 10, Room 4N246, Bethesda, MD 20205

In recent years, recordings from human intracranial electrodes have suggested that the P300 component of the event-related brain potential (ERP) may be generated by limbic structures (i.e., pes, uncus and amygdala). To determine if this activity is the source of the scalp-recorded ERP, we evaluated patients with resections of the left or right anterior temporal lobe. We reasoned that, if P300 were generated exclusively by limbic structures, removal of one of two symmetrical neural sources should dramatically alter the scalp-recorded P300.

Eight patients with a left temporal lobectomy (LTL) and eight with a right temporal lobectomy (RTL) were matched with normal controls (NC). The resection included the anterior portion of the temporal lobe (averaging 5.0 cm for the LTLs, and 6.5 cm for the RTLs), with complete removal of the pes, uncus and amygdala and partial removal of the hippocampus (1.5-2.5 cm). All performed the standard "Oddball" paradigm under two instructional conditions: Count and Reaction Time (RT). In separate series, low-pitched tones were presented at probabilities of .10, .30 and .50. The EEG was recorded from Fz, Cz, Pz, F3, F4, C3, C4, P3, P4, T3, and T4, all referred to linked mastoid electrodes. Eye movement activity was also recorded.

P300 amplitude was inversely proportional to stimulus probability in all three groups and larger P300s were elicited in the RT condition. P300 activity was essentially identical in RTLs and NCs. LTLs, however, had smaller P300s owing to a negative shift that began about 90 msec after stimulus onset. The latencies of N2 and P300 were delayed by approximately 50 msec in the RTLs compared to the LTLs. The within-group hemispheric comparisons revealed that, in most cases, there was a slight asymmetry in P300 amplitude in the Count and RT tasks with larger potentials present over the right hemisphere in both lobectomy groups and NCs. In contrast, the N1 component was asymmetrical with the largest potentials occurring over the operated hemisphere.

In the data collected to date, we have not observed any consistent hemispheric asymmetries in the distribution of P300 that distinguish either the LTLs from the RTLs, or either group from the NCs. The current data do not support the hypothesis that P300 activity is generated exclusively by limbic structures.

251.6 SEQUENTIAL STIMULUS EFFECTS ON THE LATE POSITIVE COMPONENT OF THE AUDITORY EVENT-RELATED POTENTIAL (ERP) IN MONKEY. D. L. Arthur* and A. Starr. (Spon: T. O'Connor). Depts. of Psychobiology and Neurology, California College of Medicine Irvine, CA 92717.

The late positive component of the human ERP, P300, is an endogenous component whose amplitude is related to at least three interactive factors; 1) task relevance, 2) stimulus probability and 3) the precise sequence of preceding stimuli. We previously reported (Arthur, D. L. and Starr, A., Science, 223:186, 1984) a long latency (300 msec), vertex positive component of the ERP recorded from monkeys (Macaca nemestrina) which was present only when the eliciting stimulus was relevant to the learned discrimination task. The amplitude of this component varied inversely with stimulus probability and was dissociable from motor responses.

To further assess the correspondence between the late positive component elicited in monkeys with the human P300 component, we are investigating the effects of the precise sequence of stimuli preceding the eliciting (CS+) stimulus. On each trial a high pitched (CS-) and low pitched (CS+) tone are equally likely to occur. Single trial EEG recorded from the vertex is digitized on line and stored on disk while the animals perform the discrimination task. Single trial data is subsequently sorted and averaged according to the precise sequence of stimuli preceding the CS+. Preliminary results indicate that when the eliciting tone (CS+) is preceded by a run of non-target (CS-) tones (eg., AAAAB), the late positive component is larger in amplitude than when the tones did not change (eg., BBBBB). This trend resembles the relationship between ERP amplitude and stimulus sequence seen in the human P300 component.

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251.7 NORMAL LATENCY VARIABILITY IN THE AUDITORY EVENT-RELATED POTENTIAL. H.J. Michalewski*, D. Prasher* and A. Starr. Department of Neurology, University of California, Irvine, CA 92717.

Traditional averaging procedures may sometimes obscure features of the event-related potential (ERP), such as component variability. We have attempted to estimate the latencies of N1, P2, N2, and P3 in the ERP waveform on a trial-by-trial basis. In addition to measures of component variability, the relationship among components to the prediction of P3 latency was examined.

Auditory ERPs were collected from a group of normal (N = 12) individuals in an oddball paradigm. Subjects were required to detect an occasional high frequency tone (640 Hz) interspersed among a sequence of low frequency (440 Hz) tones. Scalp potentials were recorded from midline sites Fz, Cz, and Pz referenced to linked earlobes. Each tone trial was digitized and saved to disk. Averages were computed to target (high) tones (N = 60). The single trial analysis used a modified version of the Woody correlational-template technique to identify the major peaks of the ERP. Separate templates, derived from the individual peaks of the average waveform, were used to define component shapes. Estimates of peak latency were computed separately for each component successively.

Based on the results of the Woody analysis, the following mean peak latencies (msec) and (standard deviations) were found: (1) for Pz, N1 = 91.1 (20.2), P2 = 170.2 (27.1), N2 = 242.5 (51.9), and P3 = 324.3 (52.1); (2) for Cz, N1 = 88.9 (16.4), P2 = 166.8 (24.1), N2 = 246.9 (55.5), and P3 = 341.1 (59.9); and (3) for Fz, N1 = 89.9 (16.8), P2 = 172.1 (26.0), N2 = 244.4 (53.9), and P3 = 327.3 (58.9). Analysis of peak variances indicated that the variance of N1 was significantly less than the variance of P2, the variance of P2 was less than either the variance of N2 or P3, but that the variance of N2 was not different from the variance of P3.

The contribution of various peaks to the prediction of P3 latency was evaluated in a series of regression analyses. Predictors of P3 included N1, P2, and N2 considered separately or in combination. Over 60% of the variance of P3 was accounted for by N2. Including N1 and/or P2 did not add to the prediction of P3. Measures of variance and the relationships among peaks may add useful information in characterizing the ERP components.

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251.8 STATISTICAL COMPARISON OF PRINCIPAL COMPONENT ANALYSES OF EVENT-RELATED POTENTIAL DATA. D. Guthrie*, R.J. Strandburg, J.T. Marsh, W.S. Brown*, R.F. Asarnow*. Dept. of Psychiatry, UCLA Medical School, Los Angeles, CA 90024.

Principal Components Analysis (PCA) is commonly used in ERP research to identify components underlying the averaged waveform, provide an objective means of quantifying their presence in each ERP, and, thereby, reduce the number of variables to be examined in subsequent analyses. When comparing ERP data from independent groups, subjects are typically combined in a single PCA so that factor scores will be metrically equivalent across groups. This assumes, however, that the subject groups differ only in the degree to which each factor is present in their ERPs (component amplitude) and not in the factor structure (number, latency and duration of components).

We will present a statistic for determining the degree of overlap between factor structures from separate subject groups (or electrodes or experimental conditions) and will suggest applications of this statistic to other problems in ERP research.

Essentially, the angle in multi-dimensional space between eigenspaces is computed for any two factor structures (comparison of "factors" or basis vectors). As there is no known sampling distribution for such a statistic, an empirical distribution is computed using a series of random subject allocations. Factor structure similarity is then assessed by observing where the value obtained from the actual subject grouping falls in this simulated distribution.

This statistic can be used to detect latency differences, determine the appropriate number of extracted factors, and identify factors which might reflect unique properties of each group.

This process will be illustrated with ERP data from our previously reported studies of schizophrenic, age-matched normal, and younger normal children (Strandburg et al., Electroenceph. clin. Neurophysiol., 1984, 57: 236-253).

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251.9 ATTENTION SELECTIVELY MODULATES PARALLEL VISUAL PROCESSES. G. Schechter* and E. Callaway. Langley Porter Psychiatric Institute, University of California, San Francisco, California 94143.

The complexity of the visual environment necessitates continuous selection of only relevant information for processing. Selective attention is influenced by both the physical parameters of the sensory stimuli and the cognitive strategies employed by the perceiver. Characteristics of visual stimuli have been defined in terms of spatial frequency (low-high), size (large-small), and level of organization (global-local). Although these distinctions overlap, it is generally assumed that information of different spatial frequencies, sizes or levels is processed in parallel channels extending from the retina to the cortex. In addition to stimulus characteristics, evidence suggests a crucial role for attentional modulation of incoming stimuli.

Evoked potential (recorded at vertex) and reaction time responses to identical stimuli, large letters composed of small letters, obtained from 28 normal adults were compared under instructions to attend to designated large letters, small letters, or both.

(Examples)

Z	Z	H	H	
Z	Z	H	H	
ZZZZZ		HHHHH		
Z	Z	H	H	
Z	Z	H	H	

A repeated measures analysis of variance was used to evaluate the effects of stimulus type, attention condition, and their interaction. Results showed a significant stimulus effect; both P3 latency and RT were faster to large compared to small targets. A significant attention effect indicated that response times to attended stimuli were speeded while those to nonattended stimuli were slowed. Thus, both processing facilitation of relevant information and processing inhibition of irrelevant information occurred. A significant stimulus-attention interaction revealed stronger effects of attention on large than on small stimuli, indicating that global processing was more ameniable to cognitive modulation. All effects were greater for RT than for P3 latency recorded from the vertex, and EP components occurring earlier than 300 msec after stimulus onset showed no significant changes with attention condition. The pattern of results suggests a late time course for this cognitive activity and implicates higher cortical centers as the locus.

This study demonstrates that attentional modulation, quantified as response facilitation or inhibition, in processing selected elements from a complex visual display occurs in normal adults. Results show that information processed in parallel is differentially sensitive to attentional deployment. The stimulus-attention interaction suggests independent functions for the parallel systems that are consistent with neuroanatomical, neurophysiological and behavioral evidence.

251.10 MULTIPLE GENERATORS OF THE P3 POTENTIAL: IMPLICATIONS OF HIPPOCAMPAL EPILEPTIC SPIKES. I.Altafullah*, E.Halgren, J.M.Stapleton, & P.Crandall. Brain Research Inst. & V.A. Southwest Reg. Epilepsy Ctr., UCLA, Los Angeles, CA 90024

The 'P3' is an endogenous scalp potential typically evoked with a latency of about 300 ms by rare stimuli in a detection task. It has usually been assumed that the P3 is generated by synchronous activation of specific neocortical synapses. However, we found (Science 210: 803, 1980) large polarity-reversing field potentials in the human medial temporal lobe (MTL) and changes in MTL neuronal firing simultaneous with the scalp-P3.

We compare here the MTL voltage distributions of epileptic interictal spike-waves (IIS) with those of the MTL-P3 recorded from the same electrodes during an auditory oddball task. MTL electrodes were implanted in 12 patients to localize the onset of seizures. Field potentials (0.1 to 100 Hz) were recorded from Fpz, calvarial electrodes at Fz and Cz, and from 19 to 114 depth contacts, all referred to the nose tip. IIS from each patient were averaged together. 9 of the 12 patients displayed a stereotyped IIS with an initial focal spike, reversing polarity between amygdala and hippocampus, followed by a more diffuse MTL slow wave lasting 300-600 msec. This typical slow wave (TSW) was small and positive in the most superficial leads, increased in amplitude as the hippocampus was approached and reversed polarity over short distances in the deepest hippocampal leads. Largest amplitude was usually in the anterior hippocampus. In the sites observed to date, this distribution has been essentially identical in polarity and relative amplitude to that of the MTL-P3, suggesting that the two are generated by synaptic activation of the same MTL neurons. A positive potential was reliably recorded at the vertex during the TSW, presumably representing passive volume conduction of the TSW to the scalp. Comparison of relative amplitudes of the surface positivity with the TSW amplitude, and relative amplitudes of the scalp-P3 with the MTL-P3, indicate greater attentuation of the TSW than the MTL-P3. In multicontact electrodes the positivity during the MTL-P3 does not drop off in the lateral cortical contacts, as it does for the TSW and often rises again in the superficial leads. Thus the scalp-P3 appears to represent activation of the MTL as well as other, probably more superficial, generators.

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251.11 MAPPING INFORMATION PROCESSING IN THE MONKEY CORTEX WITH EVOKED RESPONSES FROM BIPOLAR TRANSCORTICAL ELECTRODES. Richard K. Nakamura, Richard Coppola, and Allan F. Mirsky. Lab. of Psych. and Psychopath., NIMH, Bethesda, MD 20205.

We are attempting to map information processing across the cerebral cortex of the monkey based on cortical evoked responses. Previous analyses have suffered from lack of sensitivity, reliability, replicability, or interpretability. Such problems are reduced in this investigation by a variety of strategies, the most important being: a) arrays of bipolar transcortical (surface to depth) electrodes, b) a highly controlled behavioral task with several variations, and c) a large number of trials (2000) per day.

Electrodes are placed over the cortical surface of one hemisphere with sulcal impressions in the concavity of the skull as landmarks. The basic task is a go/no-go visual pattern discrimination that is reversed daily. The visual stimuli are presented for 100 ms on a shutter controlled screen. Task variations include: changing visual patterns, manipulating brightness, using an auditory stimulus, requiring all-go responses, and altering the percentage of correct go responses that are rewarded. Evoked responses recorded from electrodes in an animal performing this task are bandpassed at .1 to 100 Hz and sampled at 200 times/sec.

In the three monkeys tested thus far we found high reliability over time in signals from individual electrodes. Evoked response patterns were also replicated in both form and response to task manipulation at corresponding brain sites across animals. Responses at individual sites were sensitive to different aspects of the task including: stimulus pattern, stimulus meaning, stimulus modality, motor response, and reward delivery. Responses measured at different sites showed considerable variation even when they were only a few millimeters apart. In general, electrodes in the occipital and inferior temporal cortex were sensitive only to visual information whereas parietal electrodes anterior to the visual system were sensitive mainly to the motor response or reward delivery. Evoked responses in the precentral gyrus reflected not only the motor response but stimulus and reward delivery as well. Prefrontal electrodes showed the greatest variety of responses.

The power of this approach lies in its potential to summarize information processing across all the cytoarchitectonic regions of the cortical mantle simultaneously. Our results to date suggest the necessary sensitivity, reliability, and replicability to fulfill this potential.

251.12 EVOKED POTENTIAL ANALYSIS OF EVENTS UNDERLYING REACTION TIME DIFFERENCES IN THE MONKEY. Richard Coppola, Nora J. Besansky*, and Richard K. Nakamura. Lab. of Psychology and Psychopathology, NIMH, Bethesda, MD 20205.

Reaction time (RT) is a ubiquitous physiological and behavioral measure that must often be treated as univariate. The spatial and temporal sensitivity of our system for mapping cortical information processing in the monkey allows differentiation of the cerebral events underlying an animal's RT. This is clearly illustrated by a double dissociation of effects following two different manipulations that reduce RT.

First, RT can be reduced by increasing the brightness of a visual trigger stimulus. However, the relative contributions of the various CNS processing stages to the overall changes in RT with brightness have not yet been firmly established. On our go/no-go visual pattern discrimination task, an 8:1 brightness increase in the visual patterns produced a savings of approximately 30 ms in an overall RT that averaged approximately 300 ms. An analysis of the evoked cortical responses indicates that the earliest clear peaks, which appeared at 89 ms to the dimmer stimuli occurred 24 ms earlier to the brighter stimuli. This suggests that 80% of the RT difference is attributable to precortical delays which occur in the first quarter of the total RT. Since peaks appearing after 100 ms show the full RT difference, the entire RT savings is accounted for by processing that is completed in the first third of the total RT.

Second, RT can be reduced by simplification of the decisions required before a response. We compared reaction times when an animal is required to do the standard go/no-go task to those when the animal nas to 'go' on all trials. This go task yielded a 20 ms savings in RT. The latencies to all the identifiable evoked response peaks prior to the motor peak, were within 3 ms of each other. On the other hand, the motor peak reflected the full 20 ms difference. The last identifiable peak before the motor peak is at 180 ms. Thus all the RT savings in this case occured in the last third of total processing time. In contrast to the case of brightness manipulation where larger stimulus-related peaks were associated with faster reaction times, in this case, smaller stimulus related peaks were associated with faster reaction times.

251.13 EVOKED POTENTIAL ANALYSIS OF VISUAL INPUT TO PRECENTRAL GYRUS (MOTOR CORTEX) OF MONKEYS. Nora J. Besansky* and Richard K. Nakamura (Spon.: C. C. Duncan-Johnson). Lab. of Psychology and Psychopath., NIMH, Bethesda MD 20205.

While studies have shown that the precentral gyrus contains neurons that respond to visual and auditory stimuli, there is little agreement about the significance of these responses. We have implanted arrays of transcortical electrodes in monkeys trained to do a go/no-go visual discrimination task. Evoked responses from electrodes in the precentral gyrus of the monkey showed a characteristic set of waves. The earliest component, which begins at approximately 65 ms, was tightly time-locked to the onset of the stimulus and appeared in exactly the same form and amplitude whether or not the animal made a 'go' response. This component first appeared 5-10 ms before any detectable activity in visual cortex and had a polarity opposite to that seen in the visual cortex. A later component predicted and was time locked to the response of the animal. This started by 150 ms and, on go trials, peaked just after the response which occurred between 250 and 330 ms. Although the sensory component of the evoked response appeared widely in the precentral gyrus, it was very weak or nonexistent in both postcentral gyrus and prefrontal cortex.

Varying the brightness of the stimulus affected both the amplitude and latency of the sensory component in the precentral gyrus as well as in the visual cortex. Unlike visual cortex, however, the precentral gyrus was not sensitive to changes in the stimulus pattern. Further, manipulation of task parameters that change the meaning of the stimulus could also influence activity in visual areas without doing so in precentral gyrus. The differences between the patterns of evoked responses in visual cortex and precentral gyrus of latency, form, and response to manipulations of meaning raise the possibility that the signal in precentral gyrus was derived from a noncortical visual area such as the optic tectum.

One monkey that has been trained to respond to auditory stimuli showed a sensory component in the precentral gyrus similar to the one evoked by visual stimuli but at a 20 ms latency. This appeared only after the animal had been trained to respond quickly to the auditory signal.

These results suggest that the precentral gyrus receives behaviorally relevant sensory information and may derive this information from a subcortical source. The function of the sensory input may be to prepare the motor system for a fast stimulus-triggered response.

251.14 MONKEYS WITH LESIONS OF HIPPOCAMPUS AND AMYGDALA EXHIBIT EVENT-RELATED BRAIN POTENTIALS THAT RESEMBLE THE HUMAN P300 WAVE. K.A. Paller, S. Zola-Morgan, L.R. Squire, and S.A. Hillyard. Depts. of Neurosciences and Psychiatry, U.C.S.D. Sch. of Med., and VA Medical Center, La Jolla, CA 92093.

Perceptual and cognitive events in humans are reliably correlated with neurophysiological measures known as event-related brain potentials (ERPs). Among these, the P300 wave has been considered to reflect information processing related to expectancy, orienting, and memory. Medial temporal lobe brain structures have been implicated as possible sources of this electrical activity. To improve our understanding of the neural bases of these potentials and the associated cognitive processes, similar potentials have been investigated in the monkey.

Eight Cynomolgous monkeys (Macaca fasicularis) had screw electrodes chronically implanted at selected skull sites. "Oddball" sequences of randomly ordered 1450 Hz (90%) and 300 Hz (10%) tone bursts having no conditioned significance were presented with a one sec interstimulus interval. ERPs to the rare tones included a prominent positive wave between 150 and 450 msec after stimulus-onset, of maximal amplitude at central midline sites. Similar ERPs were elicited by stimuli that resembled monkey vocalizations. During other sequences in which two stimuli were equally probable, the amplitude of the late positive wave varied systematically in the manner of the human P300 component; the positivity was largest when the evoking stimulus differed from previous stimuli. Four monkeys were trained in a tone-frequency discrimination task. A similar late positive ERP was elicited by the task-relevant rare tones under these conditions, and its amplitude gradually habituated when manipulandum and reward apparatus were removed.

Similar ERPs were recorded from humans under comparable conditions. Cross-species parallels suggest that the monkey late positive waves and the human P300 waves may reflect homologous neurophysiological processes.

Each of five monkeys with bilateral medial temporal lobe resections exhibited late positive ERPs, although waveforms were altered in some conditions. ERP differences were analyzed in between-group comparisons (three intact monkeys and three lesioned monkeys) and within-group comparisons (two monkeys pre- and post-surgery). These data indicate that at least some late positive activity in the monkey is not dependent on the hippocampus or amygdala, thus supporting the hypothesis that these brain structures are not the exclusive generators of the P300 in humans.

251.15 ENDOGENOUS COMPONENTS OF EVENT RELATED POTENTIALS OF THE CAT BRAIN. C. Başar* and E. Başar*, Institute of Physiology, Medical University Lübeck, 2400 Lübeck, FRG

Several investigators have shown that human endogenous event related potentials (ERPs) can be related to brain's matching and decision-making processing since its discovery by Sutton et al. (Science, 150, 1187, 1965). However, only few investigators have started investigations with animal experiments to establish a correspondence with human ERPs. (Buchwald, J.S., and Squires, N.: In. Woody, C.D. (Ed.): Conditioning. Plenum Press, New York, 1982). In our earlier reports, we have been able to show that the sensory evoked potentials can be described as a superposition of the enhanced EEG in various frequency channels. For example, the cortical auditory evoked potential of the cat is composed of enhanced damped oscillations in 1-3.5 Hz, 3.5-8 Hz, 8-15 Hz, 15-20 Hz and 40 Hz frequency ranges. This working hypothesis was developed by using a combined EEG-ERP algorithm and the use of response adaptive digital filters which allowed an efficient signal/noise extraction in the analysis of single ERP sweeps. (E. Başar: EEG-Brain Dynamics, Elsevier/North-Holland, Amsterdam, New York, 1980). In order to analyze the endogenous components of ERPs in 5 chronically implanted and freely moving cats we have used the odd-ball paradigm by acoustical stimulation of cats with 1550 Hz (rare) tones presented randomly amongst 1500 Hz (frequent) tones. The phenomenon of enhancement has been also observed in ERPs during the experiments with the odd-ball paradigm in the same frequency bands as the sensory evoked potentials. However, the duration of the enhanced reactions in the 0.5-3.5 Hz, 3.5-8 Hz, 8-13 Hz and 40 Hz are delayed and prolonged in comparison to the reaction to simple 1500 Hz stimuli. For example, the 3-8 Hz cortical responses to simple auditory tone bursts of 1500 Hz usually have the shape of a wave packet with 2 or 3 damped oscillations whereas stimulation with complex sequence (especially rare tones) elicit ERP reactions having more than 2 or 3 oscillations (sometimes up to 6) in the same frequency range. The damped oscillations are prolonged also in the 0.5-3.5 Hz, 8-13 Hz and 40 Hz frequency ranges. Principially, similar recordings have been established in subcortical structures as reticular formation, hippocampus and medial geniculate nucleus of the cat brain. We tentatively conclude that during matching and decision-making processes the reactions of the delta, theta and alpha enhancements have longer durations in comparison to EP-enhancement phenomena elicited with simple stimulation.

251.16 EFFECTS OF STIMULUS RELEVANCE AND PROBABILITY ON NEURONAL ACTIVITY OF LIMBIC STRUCTURES IN RABBITS. N. Stolar*, S.P. Sparenborg, M. Gabriel., R. Markese*, E. Donchin. Dept. Psychol., Univ. Illinois, Champaign, IL, 61820.

This study continues the assessment of a conditioning paradigm with rabbits as a model system for study of the neural substrates of the P300 component of the event-related potential (ERP) in humans.

Past work has shown surface and intracranial ERPs that covaried, as P300, with the probability and significance of the eliciting stimuli (Gabriel et al., Neurosci Abst., 347.28, 1983). Here, the neuronal activity recorded from the structures yielding the macropotentials is described to provide information on the intracranial origins of, and possible regional differences in the neural response.

Rabbits with unit recording electrodes were trained to move in an activity wheel to avoid a footshock whenever they heard a tone (CS+). They also learned to ignore a second tone (CS-) that was not predictive of the footshock. 120 trials (60 with each stimulus, in a random order) were presented daily until a criterion of discriminative performance was attained. Following criterion, three additional sessions were given in which the probabilities of the CS+ and CS- were, respectively, .5/.5, .2/.8, and .8/.2. The order of the last two sessions was counterbalanced.

Neuronal records obtained from the anterior cingulate cortex (area 24, N=10), the posterior cingulate cortex (area 29, N=7), the dentate gyrus (N=6), the medial dorsal thalamic nucleus (N=4), and the anterior ventral thalamic nucleus (N=5) manifested the development of a "target effect", i.e., greater neuronal discharge to the CS+ than to the CS-, during the course of training. In addition, all records showed increased firing to the CS- when it was presented rarely, and decreased firing when it was presented frequently, relative to the discharges elicited by the standard CS-. The cingulate cortical but not the thalamic records also manifested an increased discharge to the rare CS+, relative to the standard CS+. These results essentially replicate those previously obtained with macropotentials and they indicate that activity analogous to the P300 is manifested by neurons in the cingulate cortex, related thalamic nuclei, and the hippocampus. Observed regional differences suggest that stimulus infrequency can augment the cortical response to a target stimulus, whereas such augmentation does not occur in the thalamus.

251.17 IMAGERY MODULATION OF EXPERIMENTAL TONIC HUMAN PAIN: BEHAVIORAL AND CORTICAL POWER SPECTRUM ANALYSES. A.C.N. Chen, S.F. Dworkin* & L. Leresche*, Pain Center, U of Washington, 98195.

Late components of the evoked potentials are shown to be functional correlates of induced pain in man. But, due to repetitions of noxious stimuli with very brief duration, such phasic pain is known to lack a natural analogue. To evaluate the brain's responses to nociception of clinical relevance, we studied the cortical power spectrum (CPS) during longer lasting diffuse pain using the cold-pressor test as a tonic pain model. We aimed to examine whether change in CPS components is related to subjective painful experience and whether such pain experience is associated with lateralized EEG.

EEG was recorded for both left and right hemispheres, at F3-P3 and F4-P4 with linked ear reference, in 25 healthy male subjects (age 24±3). EEG was cut off at 100 Hz, sampling at 250 Hz, and FFT analyses resulted in power density for delta, theta, alpha and beta bands. Each study stage was a recording of 10 epochs/spectrum, 6 spectra/min for a total of 5 min CPS. Three stages of EEG were recorded: Baseline, 1'C Cold-Pressor Test, and Imagery Modulation in combination with the cold-pressor. Also gathered were Visual Pain Analogues, State Anxiety Scale and the McGill Pain Questionnaire. Before the recording, subjects completed scales of Trait Anxiety, Fear of Pain, Tellegen's Absorbance Score and Rosenbaum's Self-Regulation Inventory.

Behaviorally, there was, unexpectedly, a distinct bimodal distribution of subject responses to the cold-pressor test: a pain tolerance group (PT=16) could tolerate the entire 5min test, while a pain sensitive group (PS=9) held their hands in the icewater for only a mean of 1.5±0.5 min ($t=13.4$, $p<.0001$). These two groups differed at Baseline on measures of State Anxiety (PT=31.8, PS=37.8; $t=1.92$, $p<.07$) and Fear Score (PT=37.8, PS=47.0; $t=2.04$, $p\sim.05$). Anxiety Score was increased during the Cold-Pressor Test (PT=38.1, PS=40.7) and reduced to Baseline levels during Imagery Modulation (PT=31.6, PS=36.6). These changes were associated with reports on Visual Pain Analogues (PT=41.3, PS=79.1) during the Cold-Pressor and a slight reduction in Imagery Modulation (PT=38.1, PS=64.4). No laterality difference in CPS was observed in Baseline, Cold-Pressor Test, or Imagery Modulation stages. α-desynchronization was observed (24% reduction) under the Cold-Pressor Test and fully recovered to Baseline values in the Imagery Modulation stage. No change in theta power was observed. In contrast, delta power increased by 65% for the PT group but not changed for the PS group during the Cold-Pressor, and then significantly decreased in the Imagery Modulation for both.

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251.18 THE CONTINGENT NEGATIVE VARIATION IN HYPERKINETIC CHILDREN. R.T. Pivik and F. Bylsma*. Psychophysiol. Lab, Dept. Psychiat. & Sch. of Psychol., Univ. of Ottawa, Ottawa, Ontario, Canada K1H 8L6.

The contingent negative variation (CNV) is thought to reflect mechanisms involved in attention and arousal--functions considered to be impaired in hyperkinetic (Hk) children. In the present investigation these responses were examined in 16 Hk and 7 age-matched normal control children.

Subjects were unmedicated 8-12 year old males with I.Q. scores in the normal range. Diagnoses of hyperactivity were based on psychological evaluations and scores of $\geq$15 on the hyperactivity scale of Conner's Parent and Teacher Behavioral Rating Scales. CNV responses were recorded from a central derivation (Cz referenced to linked mastoids). The skin under these placements was scratched to eliminate contamination of CNV potentials by slow skin potentials. Vertical eye movements were also recorded and responses associated with such activity were excluded from analysis. Seventy pairs of 1500 Hz tones (70 dB; 200 and 100 msec duration, respectively) separated by 4 seconds with inter-pair intervals of 11 seconds, were delivered via an ear insert microphone. The subjects were aware of the timing of the tones and were instructed to press a handheld button as soon as the second tone occurred. EEG activity was digitized (200 samples/sec) and deviations from a 1 second pre-warning stimulus (A1) baseline determined for the 4 second inter-stimulus interval.

All subjects exhibited sustained CNV responses and mean response amplitudes for the 4 sec inter-stimulus interval did not differ significantly across groups [$\bar{X}$ amplitudes (μV): C=5.8 ± 3.0; Hk=5.4 ± 2.7). Reaction times to the button-press response were slower and more variable for Hk than C children [$\bar{X}$ response times (msec): Hk=457.8 ± 158.5; C=419.2 ± 90.4]. CNV responses in unmedicated Hk children are comparable in amplitude to those of non-Hk controls. It remains to be determined whether the enhanced variability of the associated motor responses reflects less stable CNV-related mechanisms in Hk children, within group heterogeneity, or a combination of these factors.

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251.19 UNILATERAL 6-OHDA LESIONS IN THE NIGROSTRIATAL SYSTEM OF WISTAR RATS: ELECTROCORTICOGRAPHIC EFFECTS AND THEIR PHARMACOLOGICAL MODULATION. A.C.Rossi* R.Maj*, G.Pagani*, L.Pegrassi* and M.Buonamici* (SPON: P.F. Spano). Biological R.& D., Farmitalia C.Erba Nerviano, Italy 20014.

The method of unilateral 6-OHDA lesions in the nigrostriatal system of the rat, as first described by Ungerstedt U., et al.(Brain Res., 24: 485, 1970), is a quite aknowledged experimental model for studies of different functional states in nigrostriatal dopamine system. Anyway no information seems available on the electroencephalographic consequences of this localized 6-OHDA lesion.

We have reproduced Ungerstedt's method in 12 male Wistar rats weighing 200-210 g. Stereotaxic lesions were placed by injecting 12 mcg of dissolved 6-OHDA in a volume of 4 mcl over 4 min through a Hamilton cannula (diameter 0.5 mm) inserted just rostral to the right substantia nigra of 6 animals. Control injections of the solvent alone were made in the six remaining animals as described above. 42 days after the injections the animals were implanted with chronic electrodes for electrocorticographic (ECoG) recordings as reported elsewhere (Buonamici, M., et al., Neuropharmacol., 21: 825, 1982). ECoG recordings occurred 49 and 56 days after the intrastriatal injections.

The power-spectral analysis of ECoG recordings showed the quite unique appearance of high-frequency waves of 30-40 Hz (HFW) exclusively emitted by the affected cerebral hemisphere of lesioned animals. Systemic treatments with dopamine or acetilcholine agonists and antagonists did show that HFW temporarily disappeared after apomorphine or atropine. Moreover, the physostigmine-induced increase of HFW was promptly reversed by apomorphine, which in addition eliminated HFW for about 1 hour. The exploration of other pharmacological treatments is under way.

REGIONAL NEUROPHARMACOLOGY

252.1 PHARMACOLOGICAL MANIPULATION OF THE SUBSTANTIA INNOMINATA-CORTICAL CHOLINERGIC PATHWAY. G.L. Wenk. Department of Psychology, The Johns Hopkins Univ., Baltimore, MD 21218.

Neocortical cholinergic afferents originating in the substantia innominata (SI) are consistently affected in the brains of patients with Alzheimer's Disease (AD). The design of effective pharmacotherapies for the treatment of AD will be assisted by a thorough knowledge of neuronal systems which regulate the activity of the cholinergic neurons in the SI. Male, Sprague-Dawley rats received a unilateral microinfusion of a pharmacological agent (1.0 ul) into the SI via a chronic indwelling cannula. In vitro sodium-dependent high affinity choline uptake (SDHACU) was determined in the frontal cortex 50 minutes after the infusion to indicate the in vivo activity of cholinergic cells in the SI. SDHACU in the cortex ipsilateral to the infusion was compared to SDHACU in the contralateral cortex. The results are summarized in the table below.

AGENTS THAT ALTER FOREBRAIN CHOLINERGIC ACTIVITY

Decreases SDHACU	Amount Injected (nmoles/ul)
Muscimol	44
	88
Leu,Met-Enkephalin	40
Imipramine	100
Apomorphine?	16
Increases SDHACU	
Glutamate	20
Does not affect SDHACU	
Saline vehicle, isotonic, pH 7.4	
LSD	10
Serotonin	1000
Clonidine	10
Muscimol	11
Isoproterenol	100

These results add support to immunocytochemical evidence for the presence of GABAnergic, enkephalinergic and dopaminergic terminals in the SI and suggest possible neuronal systems which may influence the activity of the basal forebrain cholinergic system. (Supported by grant DAMD 17-82-C-2225)

252.2 LONG-TERM AMPHETAMINE TREATMENT REDUCES THE NEURONAL RESPONSE TO IONTOPHORETIC DOPAMINE AND THE ELECTROCHEMICAL RESPONSE TO AMPHETAMINE IN THE NEOSTRIATUM. R. L. Wilson*, K. Kamata, K. D. Alloway and G. V. Rebec (SPON: R.M. Wightman). Depts. Chem. and Psychol., Indiana Univ., Bloomington, IN 47405.

The behavioral response to amphetamine depends, in large measure, on the integrity of dopamine (DA)-containing neurons that project from the substantia nigra to the ipsilateral neostriatum. With long-term administration, certain components of this response are enhanced (Rebec, G.V. et al., Psychopharmacology, 77:360, 1982) and there is a corresponding change in the activity of both nigral (Kamata, K. and Rebec, G.V., Neuropharmacology, 22:1377, 1983) and neostriatal (Alloway, K.D. and Rebec, G.V., Brain Res., 273:71, 1983) neurons. In the present series of experiments, we began an investigation of the mechanisms underlying the neurophysiological changes produced by long-term amphetamine treatment in the neostriatum of the rat.

In one study, iontophoresis was used to investigate the response of neostriatal neurons to DA following twice daily injections of saline or 5.0 mg/kg d-amphetamine for 6 days. DA was applied at regular intervals in increasing ejection currents to glutamate-activated neurons. In saline controls, DA produced a progressive inhibition of firing rate in all 28 neurons recorded from 12 different animals. DA also suppressed neuronal activity (n=31) in amphetamine pretreated rats (n=13) but this response was significantly less pronounced. These results support the view that long-term amphetamine treatment reduces the sensitivity of postsynaptic DA receptors.

In separate groups of similarly pretreated rats, carbon-fiber electrodes were used to record electrochemical signals in response to a challenge injection of 2.5 mg/kg d-amphetamine. Pretreatment with 5.0 mg/kg significantly reduced the rise in oxidation current compared to saline-pretreated controls. In fact, pretreatment with even higher doses of amphetamine reduced the electrochemical response even more. Although voltammetric scans confirmed previous evidence (Ewing, A.G. et al., Brain Res., 249:361, 1982) that the amphetamine-induced rise in oxidation current resembled that obtained for ascorbic acid (AA), intranigral injection of 6-hydroxydopamine, which depleted neostriatal DA by more than 98%, significantly reduced the electrochemical signal in all groups. Thus, tolerance to the amphetamine-induced rise in an AA-like substance appears to be controlled, at least in part, by DA neurons.

252.3 SUBSTANTIA NIGRA PARS RETICULATA NEURONS: ENHANCED RESPONSE TO AMPHETAMINE FOLLOWING LONG-TERM TREATMENT. K. Kamata and G. V. Rebec. Dept. Psychology, Indiana University, Bloomington, IN 47405.

The substantia nigra pars reticulata (SNR) is part of a neuronal circuit that includes the ipsilateral neostriatum and dopaminergic neurons in the substantia nigra pars compacta (SNC). This circuit has been implicated in the behavioral response to amphetamine, a widely abused psychomotor stimulant. We have previously shown that whereas acute amphetamine inhibits neuronal activity in the SNC, long-term treatment with a relatively high dose (5.0 mg/kg) significantly attenuates this response (Kamata, K. and Rebec, G.V., Neuropharmacology, 22:1377, 1983). In the neostriatum, however, multiple amphetamine injections shift the neuronal response to a prolonged excitation (Alloway, K.D. and Rebec, G.V., Brain Res., 273:71, 1983). Because the neostriatum has been reported to regulate activity in the SNR, we extended our investigation to neurons in this site.

Male, Sprague-Dawley rats (300 g) were pretreated with saline or 5.0 mg/kg d-amphetamine twice daily for 6 consecutive days. On the following day, the animals were prepared for single-unit recording. In all cases, spontaneous neuronal activity in the SNR was fast: 24.94 (±2.34) spikes/sec in saline controls (n = 17) and 29.07 (±2.74) spikes/sec in amphetamine-pretreated animals (n = 19). All these neurons displayed initially positive action potentials of less than 2.0 msec duration.

In both groups, intravenous challenge injections of d-amphetamine (from 0.25 to 2.0 mg/kg) typically accelerated SNR activity. In saline controls, the increase was relatively small, never exceeding 25% of the baseline rate. Following amphetamine pretreatment, however, the increase in firing rate was significantly enhanced. In fact, a challenge dose of 2.0 mg/kg in this group routinely doubled the activity of SNR neurons. Interestingly, a small number of cells in both groups of rats (n = 5) were depressed by an amphetamine challenge but in the animals pretreated with this drug the depression of activity was also greater than in saline controls. Thus, long-term amphetamine administration potentiated the response of SNR neurons to subsequent challenge injections of this drug. These results, which parallel those obtained in the neostriatum, suggest that the SNR is an integral component of the nigro-neostriato-nigral loop that may mediate, in part, the behavioral alterations produced by multiple amphetamine injections.

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252.4 POSSIBLE SITES OF ACTION OF TRH AS A MODULATOR OF MOTOR CONTROL PATHWAYS. R. J. Anderson, G. W. Campbell* and D. K. Boyd* (Spon: J. W. Aldridge), Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Thyrotropin Releasing Hormone (TRH) has been shown to have a beneficial effect in cases of amyotrophic lateral sclerosis, (Engel et al, Lancet, 2: 73, 1983) a motor neuron disease and in some cases of spinal cord trauma (Faden et al NEJM, 305: 1063, 1981). Although the mechanism of this action is unknown, studies have shown that TRH modifies spinal and perhaps supraspinal pathways associated with motor control (Ono et al, 21: 739, 1982). The purpose of this study was to determine whether TRH would modify or reverse abnormalities in motor activity induced by selective disruption of several neurotransmitter pathways known to mediate motor control. To determine whether TRH would modify spinal inhibition, mice were treated with a subconvulsant dose of strychnine (0.425 mg/kg) which impairs the righting reflex, increases muscle activity, induces somatosensory reflex-induced seizures in 20% of the mice but is only fatal in 5%. Pretreatment with TRH (1, 10 or 100 mg/kg) i.v. immediately prior to strychnine administration did not modify the strychnine-induced deficits. The 100 mg/kg dose given alone produced ptosis and resting tremor in the animals. Since bulbospinal pathways containing norepinephrine and serotonin are well known as modulators of fusimotor and alpha motor activity in the spinal cord (Steg, Acta Physiol Scan. 61 Supp. 225, 1964), rats were given reserpine (10 mg/kg) which enhances mono-aminergically mediated motor effects such as tremor and muscle tone. Hindlimb muscle tone was measured quantitatively using a force displacement transducer. Pretreatment with a 2 mg/kg i.v. bolus of TRH followed by i.v. infusion at a rate of 2 mg/kg per hour protected rats against the effects of subsequently administered reserpine. There was no significant change in muscle tone after reserpine administration in 5 animals pretreated with TRH. Three of these were completely protected; the two others exhibited partial protection. The infusion of TRH alone had no effect on muscle tone. These results show that TRH antagonized the motor deficits induced by reserpine but not by strychnine. The data therefore suggest that the clinical efficacy of TRH may be due to modification of descending mono-aminergic motor pathways rather than enhancing the level of spinal cord inhibition postsynaptically.

252.5 REGIONAL GLUCOSE UTILIZATION AND EEG CHANGES IN THE KETAMINE ANESTHETIZED RAT BRAIN. J.French and D.Vecchio*, Neurology, Cornell University Medical College, New York, N.Y. 10021

The anesthetic properties of ketamine HCL have been described as dissociative because parts of the brain appear to be electrically inhibited by the drug while other structures appear to be activated. Several studies have recently challenged this concept and suggest instead that ketamine anesthesia is the result of epileptiform activity in the hippocampus and amygdala. The present study used the 14C-2-deoxyglucose (2-DG) method of assessing regional cerebral glucose metabolism during ketamine anesthesia to indicate which areas of brain are activated and which are suppressed. EEG correlates from these sites were then determined in a second group of animals.

Adult male rats received tail vein and carotid artery cannulas under ether anesthesia. Following 3 hrs of recovery from anesthesia, with the rats normotensive, normothermic and partially restrained, ketamine (40 mg/kg) was injected intramuscularly. Following 45 minutes of anesthesia, 0.2 mCi of [1-14C] 2-DG was injected intra-arterially. Venous blood was sampled at intervals for 2-DG specific activity. After 45 minutes, the rats were decapitated, the brains were removed, frozen and sectioned at 20 um for autoradiography with calibrated 14C standards (Sokoloff et al, 1977).

Local cerebral glucose utilization (umol/100g/min) was significantly increased and decreased in specific neuroanatomical areas ($p<.05$) in rats given ketamine (n=5) over control rats (n=6). Increases in glucose metabolism were found in the basolateral amygdala (148% of control), the paramedian zone of the parietal cortex (135%), and the CA4 region of the dorsal (134%) and ventral (126%) hippocampus. Areas in which a decrease in glucose metabolism was found included the inferior colliculus (50% of control), the medial geniculate (57%), the dorsal (58%) and ventral (63%) frontal cortex, the cerebellar white matter (71%) and the superior olivary nucleus (73%). EEG data showed a shift to higher frequencies in metabolically active areas and a shift to lower frequencies in inactive areas.

The data support the contention that ketamine has a dissociative activity. Inhibition of brain glucose metabolism and EEG in areas associated with sensory systems (medial geniculate, inferior colliculus) may explain, in part, the anesthetic action of ketamine. The activation of hippocampal and amygdalar areas may disrupt normal processing of sensory information and may be related to the cateleptoid phenomenon associated with ketamine.

252.6 EFFECTS OF CHRONIC ADMINISTRATION OF FLUPHENAZINE ON BRAIN CYTOCHROME OXIDASE AND BEHAVIOR IN MICE. L. M. Aanonsen*, R. P. Elde and G. L. Wilcox. Depts. of Pharmacology and Anatomy, Univ. of Minnesota, Minneapolis, MN 55455

The chronic behavioral effects of fluphenazine have been studied in rats (Waddington, J. D., et al. Sci., 220:530, 1983). These studies, however, have not directly addressed the location of areas of the brain that may be targets for the action of fluphenazine. This study attempts to identify affected areas in mouse brain using a histochemical procedure for the determination of cytochrome oxidase activity (Wong-Riley, M., Brain Research, 171:11, 1979).

Male Swiss-Webster mice were injected (i.m.) with fluphenazine decanoate (2.5mg in 100 µl sesame oil per mouse) or sesame oil alone (control) once per week for a period of two months. At 1, 2, 4, and 8 weeks each mouse was observed for 5 min. in an 13 cm diameter, glass cylinder and various behaviors were noted. After each period of observation, a random sample from each group was selected for the determination of cytochrome oxidase activity in the brain. Briefly, each mouse was anesthesized with 350mg/kg chloral hydrate and perfused through the left ventricle of the heart with a low pH phosphate buffered paraformaldehyde solution followed by a high pH borate buffered paraformaldehyde solution. The brains were stored in a sucrose solution until sectioning. Brains were sectioned on a freezing microtome (50 µ sections) followed by incubation at 37°C for 1.5 h in the cytochrome oxidase media. The slices were then mounted and processed following standard procedures.

Changes in cytochrome oxidase activity were quantitated in units of relative opacity using an automatic exposure control unit (model PMCBAD) on an Olympus (model BH2) microscope. Relative opacity is expressed as a ratio between the recommended exposures at different magnifications. Results:

BEHAVIOR	CONTROL	FLUPHENAZINE	SIGNIF
perioral movement	0/8	4/5	a
body twitches	0/8	3/5	a
RELATIVE OPACITY			
neocortex	7.11+.29 (6)	7.57+.33 (5)	
sustantia nigra	7.19+.35 (6)	9.49+.41 (4)	b
nucleus accumbens	6.76+.21 (6)	8.03+.04 (5)	b

a- $p<0.05$ using Chi-square; b- $p<0.05$ using 2-tailed t-test

These results indicate the utility of chronic metabolic mapping for localization of the sites of action of chronically administered drugs. The metabolic activity of two loci thought to be involved in fluphenazine action may be altered by chronic fluphenazine treatment.

252.7 DISTINCT PATTERNS OF CNS METABOLIC ACTIVITY ARE ASSOCIATED WITH THREE BEHAVIORS PRODUCED BY APOMORPHINE. A. Braun*, P.M.Carvey*, L.C. Kao*, and H.L. Klawans* (SPON: B. Diamond), Depts. of Pharmacology and Neurological Sciences, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612

The s.c. injection of varying doses of apomorphine (APO) into the rat produces a progression of behavioral responsiveness ranging from catalepsy to stereotypy. The paradoxical expression of neuroleptoid behavior (NB) induced by 0.03 mg/kg APO as opposed to classic locomotor activity (LA) 0.45mg/kg, or stereotypic behavior (SB) 1.25 mg/kg suggests a discontinuity in the pattern of CNS activation. This potential discontinuity was explored using the 2DG method. Doses of APO producing SB appeared to selectively increase metabolic activity (MA) in the posterior (cingulate) and lateral (suprarhinal) dopaminergic (DA) projection cortices and in the dorsomedial thalamic nucleus with which these are reciprocally connected. SB doses also increased activity in the primary motor and somato-sensory (SS) cortices, the reticular, VA, and parafascicular (PF) thalamic nuclei, most regions of the hypothalamus (HT), and in the red and deep mesencephalic nuclei. SB doses also selectively increased MA in the SNC-VTA-A8 complex and within the rostral striatum and its projections: N. accumbens, olfactory tubercle, anterior (dorsal) caudate-putamen (CP), anterior GP, and SNR. Doses producing LA had an equivalent effect upon the SS cortex, VA and PF thalamic nuclei, and appeared to primarily stimulate MA in the posterior limbic (pyriform and entorhinal) cortices. LA doses also stimulated MA in most forebrain limbic nuclei exclusive of HT, as well as the caudal striatum and its projections: rostral (ventral) CP, caudal CP, caudal GP, subthalamic, entopeduncular, pedunculopontine nuclei, and VL and VM of the thalamus. Doses which produced NB appeared to selectively increase MA in the orbital and medial prefrontal (anterior DA projection) cortices, as well as the midline, and anterior-medial thalamic nuclei reciprocally connected with them. The NB doses most significantly increased MA in the lateral habenula; this also represents the principal acute affect of most typical neuroleptic agents upon MA. Although no conclusions can be drawn regarding which metabolic changes represent primary excitatory or inhibitory phenomena, these data do not appear to represent a classical dose response of APO upon CNS metabolism, but appear to identify unique patterns of metabolic activity associated with distinct behavioral states induced by this agent.

252.8 THREE-DIMENSIONAL RECONSTRUCTION OF THE RAT BRAIN FROM STEREOTAXIC ATLASES. K.A.Wagner*, A. W. Toga, N. S. Kropf*, C. Levinthal, and L. C. Murrin (SPON: L.G. Leibrock). Dept. of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE 68105, Dept. of Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110, and Dept. of Biological Sciences, Columbia Univ., NY, NY 10027.

As three-dimensional computer reconstruction becomes increasingly available to the neuroscientific community, it is imperative to standardize information interchange. Several laboratories have developed different hierarchical data base systems for storage of graphic three-dimensional anatomic data from serial reconstruction (Smith et al, Conf. Proc. NCGA, 1983).

The purpose of this study was to reconstruct to three-dimensions an accepted standard for neuroscience, the stereotaxic rat brain atlas. With permissions from authors and publishers, two rat brain atlases were reconstructed, "The Rat Brain in Stereotaxic Coordinates", Paxinos and Watson, Academic Press, Australia, 1982, and "A Stereotaxic Atlas of the Rat Brain" Pellegrino, et al., Plenum Press, New York, 1979.

Atlas digitizing was performed at the Laboratory of Neuro Imaging , Wash. U. Sch. of Med. Data capture was automated by the use of a scanning digitizer. Tests in our respective laboratories have shown that it is not practical (timewise) nor desirable from the standpoint of repeatability and accuracy to manually input voluminous amounts of graphic data. Raster to vector conversion as well as the reconstructions were carried out using a modified version of the Columbia CARTOS system (Macagno et al, (1979), Ann. Rev. Biophys. Bioeng. 8: 323-351).

The importance of a three-dimensional stereotaxic atlas is the on-line availability of neuroanatomic boundaries as an established reference in three-space for reconstruction experiments. These predefined boundaries (of nuclei, etc.) will provide, through superimposition, a rapid and repeatable means of orientation and neuroanatomical structure identification throughout the entire brain. Applications include three-dimensional mapping of mu opiate receptors in rat striatum from serial section autoradiographic data.

COMPARATIVE NEUROANATOMY

253.1 IDENTIFICATION OF ELECTRORECEPTORS IN LAMPREYS Mark Ronan and David Bodznick. Department of Biology Wesleyan University, Middletown, CT. 06457

Recent anatomical and physiological evidence suggests the electrosensory system of lampreys is homologous with those of elasmobranchs, primitive bony fishes, and some urodeles. Yet, structures similar to the ampullary electroreceptors of these gnathostomes are not found in lampreys. Experimental results now indicate that the electroreceptors of adult lampreys are the long-recognized epidermal end buds. End buds are comprised of sensory and supporting cells and, in cresyl violet-stained skin sections from adult silver (Icthyomyzon unicuspis) and sea lampreys (Petromyzon marinus), appear roughly conical with the base (approximately 50 microns across) at the epidermal surface and the apex extending to the dermis. Not normally visible in living tissue, end buds can be stained by immersing adult animals in 5% methylene blue. End buds are located over the entire body, appear more densely distributed on the head than on the trunk or tail, and are often organized into short lines or small clusters.

In three adult silver lampreys, 18-27cm total length, HRP (Sigma VI) paste on the sharpened tip of a fine metal wire was introduced into 30-40 methylene blue-stained end buds on one side of the head and into an equal number of spots in skin between end buds on the other. After eight to ten days survival at 9-11°C, animals were processed with standard HRP procedures using a modified Hanker-Yates protocol. In each case, labeled fibers terminating in the dorsal nucleus (DN), the medullary electrosensory nucleus, as well as labeled cells in the anterior lateral line nerve (ALLN) ganglion were present on the end bud-injected side and nowhere else.

In addition, uni- or bilateral HRP inoculations of DN were made in six adult silver lampreys. Animals survived ten to 21 days at 9-11°C. Inoculations included most or all of DN with variable spread into underlying structures. In four cases, including one inoculation largely confined to DN, transganglionic HRP transport labeled afferents terminating on some ipsilateral end buds. Finally, end buds were located histologically in silver and sea lamprey skin samples physiologically shown to contain receptive fields of primary electroreceptive fibers in the recurrent collateral ALLN.

End buds were intially characterized as chemoreceptors with apical microvilli on the sensory cells (Johnston, '02; Fahrenholz, '36); present results confirm a later interpretation of end buds as lateralis end organs (Whitear and Lane, '81) and identify them as petromyzontid electroreceptors.

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253.2 CEREBELLAR AFFERENTS IN THE LITTLE SKATE (BATOIDEA). R. G. Northcutt and W. J. Brunken*. Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109 and Department of Physiology and Biophysics, Washington University, St. Louis, MO 63110.

The location of neurons that give rise to afferent pathways to the corpus of the cerebellum of the little skate (Raja erinacea) were determined by unilateral pressure injection or innoculation of the anterior (5 cases) or posterior (4 cases) lobe of the corpus with HRP (Sigma VI). Following survival times of 6-14 days at 11-14°C, the animals were reanesthetized and perfused transcardially with cold phosphate buffer. Brains were removed and some embedded in 25% gelatin prior to transverse sectioning (30-40μ). Sections were reacted with o-dianisidine, tetramethyl benzidine or Hanker-Yates reagent to visualize retrogradely labeled cell bodies and fibers and were subsequently counterstained with 1% neutral red or methyl green.

Unilateral cerebellar injections retrogradely labeled cells ipsilaterally throughout the superficial and central pretectal nuclei. The pathway from the superficial pretectal nucleus is the most extensive cerebellar projection aris-ing in the forebrain and includes a small crossed component. At midbrain levels, retrogradely labeled cells were seen ipsilaterally in the dorsal and ventral accessory optic nuclei and bilaterally in two midbrain tegmental nuclei. The first of these consists of large cells scattered throughout the central portion of the tegmentum, extending from the vicinity of the ruber nucleus dorsally to include some neurons in the periventricular gray; the second is a more lateral tegmental nucleus whose cells are embedded in the anterior cerebellar peduncle. This nucleus is apparently that previously identified as nucleus H (Smeets et al., 1983). At more caudal levels, retrogradely labeled cells were seen contralaterally in the inferior olive, the descending octaval nucleus, and the ventral horn of the spinal cord. Cells were labeled bilaterally in nucleus F of Smeets et al., the nuclei of the descending trigeminal tracts and the lateral reticular nuclei. These results indicate that the cerebellar corpus in skates receives extensive visual inputs directly from pretectal nuclei and probably indirectly from the optic tectum. The single largest source of afferents to the cerebellum occurs via the midbrain tegmentum whose own afferents are presently unknown. In addition, the corpus receives spinal, trigeminal, and octaval inputs as in other vertebrates. (Supported in part by NIH grants EY02485, EY03570, and NS11006.)

253.3 AFFERENT AND EFFERENT CONNECTIONS OF THE CEREBELLAR AURICLE IN THE LITTLE SKATE, Raja erinacea. Anne W. Schmidt* and David Bodznick, Department of Biology, Wesleyan University, Middletown, CT. 06457.

The auricle of the skate cerebellum is comprised of granule populations associated with the medullary lateral line lobes. The granule cells in the most rostral portion of the auricle, lateral granule mass (LG), give rise to the parallel fibers of the cerebellar crest adjacent to the medial (mechanosensory) lateralis nucleus. The remaining caudal part of the auricle, dorsal granular ridge (DGR), provides the axons of the cerebellar crest adjacent to the dorsal (electrosensory) nucleus (Boord, 1977). The projection neurons or Purkinje-like cells of the dorsal and medial nuclei have spiny dendrites ramifying within the adjacent cerebellar crest. This suggests LG and DGR may play an important role in medullary processing of lateral line information. Using HRP and cobaltous-lysine transport we have determined the afferent and efferent connections of LG and DGR.

Anterograde transport of HRP and cobaltous-lysine demonstrated that LG and DGR efferents project as mossy fibers to the cerebellar corpus bilaterally, and confirmed the efferent projections of LG and DGR to the ipsilateral cerebellar crest adjacent to the medial and dorsal nuclei. Furthermore, small well-localized injections of cobaltous-lysine revealed a regular topographic relationship between the positions of cells in LG or DGR and the level of their axons in the crest.

The LG receives afferents from the octaval nerve and mechanoreceptive fibers in the lateral line nerves.(Koester, 1983; Boord & Roberts, 1980). Retrograde HRP transport revealed additional afferents to LG from the nucleus of the lateral funiculus bilaterally, and the rostral portion of the contralateral anterior octaval nucleus. Other LG afferents come from scattered neurons of the medullary and midbrain reticular formation. The DGR receives no major cranial nerve projections. Rather, major DGR afferents are from three cell groups at the level of the cerebellar peduncles; bilaterally from a ventrolateral nucleus (Nucleus J of Smeets et al., 1983) and ipsilaterally from two cell groups at the base of the peduncle, Nucleus F (Smeets et al., 1983) and a nucleus just lateral to F. Finally, DGR receives a direct projection from a small number of spinal neurons. Nonoverlapping inputs to LG and DGR suggest very different auricular inputs to the mechanosensory versus electrosensory lateral line nuclei.

253.4 A COBALT-LYSINE STUDY OF THE RETINAL PROJECTIONS IN THE CHAIN PICKEREL (ESOX NIGER). G.T. Bazer* and S.O.E. Ebbesson. Department of Anatomy LSU Sch. of Med., Shreveport, LA 71130.

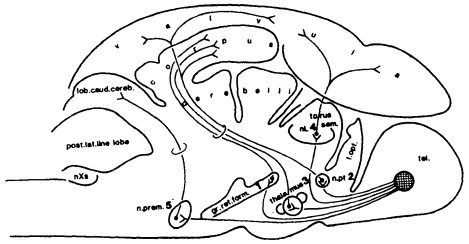
The cobalt-lysine technique for tracing neural pathways (Lazar, G. Neurosci. 3:725, 1978) has been simplified and modified from that described by Springer and Prokosch, (J. Histochem. and Cytochem., 30:1235-1242, 1982) by fixing the brain via perfusion of 3% glutaraldehyde in 0.2M phosphate buffer after perfusion with ammonium sulfide in phosphate buffer. The brains were fixed overnight and then transferred to 30% sucrose for one day before freezing and cutting on a cryostat. The sections (on the slide) were then treated with a silver intensification method (Davis, N.T., Stain Techn., 57:239, 1982) before counterstaining with 1% neutral red in 0.1N acetate buffer pH. 3.6.

The retinal projections in the pickerel were exceptionally well visualized with this method in 8 specimens. Since the pickerel is a predator depending extensively on vision, it was no surprise to find one of the best developed visual systems among teleosts. Although there are only rare ipsilateral retinal fibers projecting to the thalamus, pretectum and tectum, massive contralateral projections were found to a ventromedial optic nucleus and to most of the diencephalon and pretectum. The retinal projection to the optic tectum is highly differentiated and terminates in a selective manner in all gray strata of the tectum. The exceptional development of the visual system in this species suggests that it may become an important model for visual studies.

253.5 TELENCEPHALO-CEREBELLAR PATHWAYS IN THE BRAIN OF WEAKLY ELECTRIC FISH. T. Szabo and S. Libouban* Dept. Neurophysiologie Sensorielle, Lab. Physiologie Nerveuse, C.N.R.S., 91190 Gif sur Yvette, France.

Connections between the telencephalon and cerebellum via the rhombencephalon (Pons) are well known in mammalian brain. Such connections via the mesencephalon were first reported in 4 orders of teleost fish but have been shown to lack in Cypriniformes (Ito et al., Brain Res.,249:1, 1982).

The presence of similar pathways in the brain of weakly electric fish Gnathonemus, Pollimyrus, Brienomyrus (Mormyridae) was investigated. HRP injections into different parts of the cerebellum and the telencephalon reveal concommittant retrograde and anterograde labeling, respectively, in several structures of the brain: 1. a periventricular group of granular cells of the mesencephalic reticular formation between the posterior commissure and the oculomotor nucleus, 2. the pretectal nucleus, 3. several thalamic nuclei, 4. n. lateralis mesencephali, 5. the n. preeminentialis.



The connections of these structures, representing relay stations of the telencephalo-cerebellar pathway, are shown in the figure: the periventricular group projects to the corpus cerebelli; the epithalamic and thalamic nuclei to the corpus as well as to the valvula cerebelli; the n.lateralis projects to the valvula and the n.preeminentialis to the lobus caudalis cerebelli.

None of these structures could be identified as the paracommissural nucleus of Ito et al. The synaptic connection between the telencephalic efferents and cerebellar afferent neurons constituting these relay stations has still to be proved at ultrastructural level.

253.6 NEURAL CONTROL OF SEXUAL RESPONSES IN GOLDFISH: A DIRECT PREOPTIC-SPINAL PATHWAY. H.E. Sloan and L.S. Demski. School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

Neurons of the nucleus preoptics magnocellularis pars magnocellularis and pars gigantocellularis (terminology of Braford and Northcutt, Fish Neurobiology Vol. 2, Davis and Northcutt eds., Univ. of Michigan Press, 1983, p. 117) of Carassius auratus were labeled by placing horseradish peroxidase (HRP) in the spinal cord (SC). Pledgets of HRP were located at several different levels of transected or hemisected SC of adult animals (11-13 cm standard length). Following survival times of 3, 4 and 7 days at 26±2°C, brains were sectioned in either the transverse or horizontal plane and processed using Hanker-Yates or TMB protocols: Sections were examined with either bright or darkfield optics.

Preoptic neurons, over 100 in some fish (total of bilateral distribution), were labeled from transections of rostral SC. In contrast, fewer preoptic cells (vertebral level 5-8) were labeled from hemisections of rostral SC. In these cases most of the filled neurons were ipsilateral to the cut. Far fewer preoptic neurons were labeled following transections of SC at midvertebral (v. 14) or caudal (v. 25) levels than after higher cuts. Control experiments did not reveal heavy or moderate labelling of preoptic neurons from HRP in plasma or CSF. HRP was placed in the peritoneal cavity, on exposed SC or telencephalon and in the cerebellum or facial lobe.

The preopticospinal pathway corresponds in general to the location of a functional system controlling sperm release (SR) in goldfish. The locus of points from which SR can be elicited by electrical stimulation extends from the preoptic area to vertebral levels 3-6 (see Demski, Fish Neurobiology Vol. 2, p. 343). Some of the lowest threshold sites parallel axonal projections of preoptic neurons which course ventrolateral in the forebrain bundles and then caudad dorsal to the nucleus perglomerulosus pars lateralis, where the fibers join labeled axons of other neurons. Moreover, the loci for rostral SC transections or hemisections correspond to the probable area of SR motoneurons. Preoptic axons projecting to the lower spinal levels most likely relate to other autonomic functions. Supported by NIH grant NS 19431-02.

253.7 THE PRIMARY PROJECTIONS OF THE SENSORY COMPONENT OF THE FACIAL NERVE IN THE GOLDFISH, CARASSIUS AURATUS. R. L. Puzdrowski*. (SPON: M. S. Northcutt). Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109.

The peripheral distribution of the sensory component of the facial nerve in adult goldfish was examined using the Williams ('43) modification of the Sihler technique. The central projections of individual rami were traced with HRP. Under MS222 anesthesia, individual rami of the facial nerve were exposed and transected. A gelfoam pledget soaked with 40% HRP (Sigma VI) was placed on the proximal stump of the transected branch. After a survival time of 7-11 days at 22-26°C, the animals were reanesthetized and transcardially perfused with phosphate buffer (pH 7.4) followed by a 2% glutaraledhyde solution. The brains were sectioned transversally at 40μ and processed according to the Mesulam ('78) TMB protocol, or by the Hanker-Yates protocol.

Fibers of the facial sensory nerve were found to course in the supraorbital, palatine, infraorbital and hyomandibular trunks. As in carp, the palatine trunk was found to consist of only facial sensory fibers. Additionally, a recurrent collateral was found. The recurrent passes caudally over the VIIIth nerve and joins the posterior lateral line nerve to course caudally innervating receptors on the trunk.

The fibers of the facial sensory nerve enter the medulla as a single root and pass medially and caudally as a large bundle to terminate, for the most part ipsilaterally, in the facial lobe. Filling of either the supraorbital or infraorbital trunk, in addition to labeled fibers in the above pathway, results in a minor projection from the descending trigeminal root to the ventral most aspects of the facial lobe. Further, filling of the infraorbital trunk results in a sparse projection across the midline in the antero-ventral portion of the lobe.

The peripheral nerves were found to map somatotopically on the facial lobe. Overall, the projections follow an antero-posterior orientation with the long axis of the body tilted slightly ventrally. The supraorbital trunk projects to the rostral most and dorsal-intermediate portion of the lobe. The infraorbital and hyomandibular trunks project to the dorsal-intermediate and ventral most portions of the lobe. The projections of the palatine trunk cover a broad area of the ventral-intermediate portion of the lobe. The branches of the recurrent map broadly across the dorsal half of the lobe.

(Supported in part by NIH grants NS11006 and EY02485.)

253.8 TECTAL AFFERENTS IN CHANNEL CATFISH. M.A. Kobylack, S.C. Sharma and A.A. Dunn-Meynell. Depts. of Anatomy and Ophthalmology, New York Medical College, Valhalla, NY 10595

Horseradish peroxidase was injected unilaterally into the optic tectum of channel catfish, Ictalurus punctatus. The sources of tectal afferents were thereby revealed.

Retrogradely labelled cells were seen in both the ipsilateral and contralateral telencephalon. The superficial pretectal nuclei (P2 of Finger, '78) were also labelled on both sides of the brain. A projection was seen from the ipsilateral dorsomedial optic nucleus. Ipsilateral projections were also seen from the entopenduncular nucleus. Both the anterior thalamic nucleus and the ventro-medial thalamic nucleus projected to the ipsilateral optic tectum. Labelled cells were also seen in the deep layers of the contralateral optic tectum. Cells in both the ipsilateral and contralateral nucleus of the posterior commissure were seen to project to the tectum. Fibers were seen in the lateral geniculate nucleus ipsilateral to the injected tectum, however no cells were observed. It therefore appears that tectal cells project to the lateral geniculate nucleus, but that this projection is not reciprocal. No labelled cells were found in the cerebellum which might project to the tectum. In both the ipsilateral and contralateral medial reticular formation labelled cells were found. Labelled cells were also observed in the ipsilateral nucleus isthmi. A projection was seen from the dorsal funicular nucleus with horseradish peroxidase labelled cells only observed in the contralateral half of the brain. Finally, labelled cells were seen in the inferior nucleus of raphae. Supported by NEI-01426.

253.9 GUSTATORY CENTERS EXIST IN THE TELENCEPHALON AND THALAMUS OF CATFISH: SUPPORT FOR HOMOLOGY WITH MAMMALIAN FOREBRAIN. J.S. Kanwal, T.E. Finger† and J. Caprio. Dept. of Zoology and Physiology, Louisiana State Univ. Baton Rouge, LA 70803 and Dept. of Anatomy, Univ. of Colorado Hlth. Sci. Ctr., Denver, CO 80262†.

We report the existence of a telencephalic gustatory center and its thalamic connection in the channel catfish, Ictalurus punctatus. Although Herrick (1905) first proposed the existence of a gustatory center in the thalamus of fish, we determined its exact location. HRP injections were made in the posterior region of the thalamus. The presence or absence of retrogradely filled cells in the secondary gustatory nucleus helped to localize the thalamic taste nucleus. This ventro-medial posterior nucleus is similar to the thalamic gustatory center of mammals on the basis of topology and connectivity.

Recent studies on the general organization of the telencephalon in actinopterygean fishes suggest homologies with pallial and sub-pallial structures of land vertebrates (Northcutt, 1981). Auditory, visual and mechanosensory (lateral-line) projections to distinct targets in the telencephalon have been previously documented. After HRP injections into the thalamic gustatory nucleus we observed labelled fiber terminals and cell bodies in the ventral portion of the area dorsalis pars medialis of Nieuwenhuys (1963) in the telencephalon.

Our anatomical observations were followed by electrophysiological localization of the thalamic and telencephalic gustatory centers. The telencephalic target of the thalamofugal projection is located medially and approx. 0.2 mm anterior to the anterior commissure and extends from 1.2 to 2.0 mm below the surface. Results from these studies coincide well with the anatomical data. Few reports exist on the response properties of neurons in the telencephalon of fish. We have obtained preliminary data on single unit responses to chemical (amino acids) and tactile stimuli from cells in the thalamus and telencephalon of the channel catfish. The presence of multimodal single units in the telencephalon indicates a functional similarity between the telencephalic gustatory centers of fish and mammals.

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253.10 SPINAL CONNECTIONS WITH THE DORSAL COLUMN NUCLEUS AND BRAINSTEM IN A REPTILE. M.B. Pritz and M.E. Stritzel*. Div. Neurol. Surg., Univ. of California Irvine Med. Ctr., Orange, CA 92668.

Spinal connections with the dorsal column nucleus (DCN) and brainstem were investigated in juvenile Caiman crocodilus. Myelotomies of the dorsal and dorsolateral upper cervical spinal cord were performed under cold narcosis. Horseradish peroxidase (HRP) crystals were then applied to the exposed cut spinal cord. After survival periods of 5 to 11 days at water temperatures of 16 to 30°C, animals were anesthetized with sodium thiopental and perfused transcardially. Brains were blocked in a standard plane and tissue was processed for HRP histochemistry with tetramethylbenzidine as the chromagen.

Injection of the dorsal funiculus (DF) alone resulted in labelling of axons that terminated solely in the ipsilateral DCN. This succeeded in outlining nearly the entire extent of the DCN. By superimposing outlines of the locus of HRP labelled axon terminals, a reconstruction of the DCN was obtained. Individual DF axons were well visualized. In transverse sections, these fibers entered the DCN dorsally and in a somewhat oblique angle.

Application of HRP that extended beyond the DF and into the dorsal horn and dorsolateral spinal cord, resulted in labelled axons that terminated in the reticular formation, cerebellum, and midbrain in addition to fibers that ended in the DCN. Retrogradely labelled neurons in the reticular formation, midbrain, and diencephalon were seen only after HRP applications that extended beyond the DF.

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253.11 THE RHOMBENCEPHALIC RETICULAR FORMATION AND RAPHE NUCLEI IN A FROG (RANA PIPIENS). L. Larson-Prior and W.L.R. CRUCE Neurobiology Program, N.E. Ohio Universities College of Medicine, Rootstown, Ohio 44272.

The rhombencephalic reticular formation plays an important part in modulating both visceral and motor function. Yet, due largely to the difficulty of identifying neuronal groupings in the normal brain, it remains ill-defined in amphibians. We have chosen to define this area by experimental techniques, supplementing the analysis with Golgi and Nissl stained material.

HRP injections were made into the spinal cord, cerebellum, and mesencephalic tegmentum in adult R. pipiens. Brains were reacted with either the TMB method for HRP localization or with a heavy metal-enhanced DAB method. Spinal cord injections were confined to the dorsal and ventral quadrant of the cord.

Regardless of injection placement, small and medium sized cells labelled within the reticularis inferior (RI). There does not appear to be any discrete localization of cells by soma size, i.e. small and medium cells are randomly interspersed. No divisions could be noted with RI, whether on the basis of trajectory or cellular morphology. RI extends to the rostral edge of the VIIth motor nucleus at which point there appears to be a gap in which few cells are labelled. Rostral to this gap, at the VIIIth nerve entry, large multipolar cells of the reticularis medius (RM) are labelled bilaterally. These cells continue to the rostral edge of the Vth motor nucleus. Injections which solidly encompassed the lateral funiculus of the spinal cord showed label within the raphe. These small, round cells were clustered close to the ventricular surface. HRP injections which were placed laterally within the cerebellum, encompassing the peduncle, also resulted in label within the raphe. However, these cells were located ventral to those labelled from cord injections. Cerebellar injections also resulted in label of cells within RI contralaterally. These cells were located chiefly among the smaller elements of RI though an occasional larger cell was also labelled.

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253.12 SYNAPTIC ARCHITECTURE OF THE LAMINAR NUCLEUS OF THE RED-EARED TURTLE. A.B. Drakontides and R.H. Browner. Dept. of Anatomy, New York Medical College, Valhalla, NY 10595

The neuronal cell bodies of the laminar nucleus (LN) in the red-eared turtle are arranged in 2 to 5 layers. The analysis of ultrastructural features was limited to coronal sections taken from the medial portion of the nucleus, a region containing approximately 2 layers of somata. Observations and evaluations were restricted to the neuropil between and surrounding somata.

Ependymal cell processes, bundles of axons and axo-dendritic synapses were present between the ependyma and first layer of somata of the LN. Few myelinated fibers were present. The most characteristic feature was the presence of axonal terminals which contained clear core vesicles and large (132 nm) dense core vesicles. This type of axon terminal was often noted to form synaptic contacts with somata of the first layer of LN.

The neuropil surrounding the last layer of somata of LN contained many myelinated fibers. A great many of these fibers contained clusters and isolated ribosome-like particles. A characteristic feature evident in this region was the presence of numerous glomeruli. A glomerulus consisted of a central axonal process (approx. 9 by 5 μm) filled with clear core vesicles, that was linked to surrounding dendrites by many synaptic junctions. Often the outer core of dendrites had axonal synaptic contacts. Moreover, extremely large axonal processes, 18-20 μm in length and 3-5 μm in width, with multiple dendritic synapses were present. These processes contained clear core vesicles and in a number of instances ribosome-like particles. While they were often noted to abut on somata, synaptic contacts were not observed. A novel finding was the presence of large (18-20 μm by 3-5 μm) varicosities, filled with clear core vesicles, which were ensheathed at either end by myelin. At these ends myelin was arranged in loops similar to the paranodal regions adjacent to Nodes of Ranvier. The vesicle filled varicosity was encased by many dendrites in synaptic contact. This complex is reminiscent of enpassant synaptic apposition seen in unmyelinated axons. Whether all extremely large axonal processes were flanked by myelinated regions remains to be answered. The origin of these large axonal profiles is currently being investigated with HRP mapping.

253.13 RELATIVE BRAIN SIZE IN BIRDS. Este Armstrong and Roxanne Bergeron.* Department of Anatomy L.S.U. Med. Ctr., New Orleans, LA 70112.

Recently, an analysis of mammalian brain and body weights showed that when body size was adjusted for oxygen turnover, many taxonomic and dietary differences in relative brain size disappeared (Armstrong, Science, 1983). Birds, too, are homeothermic vertebrates, and so it was expected that metabolic rates would also influence avian relative brain sizes.

To determine the effect of metabolism on relative brain size, brain weights (E), body weights (S) and basal metabolic rates (BMR) were collected from published reports. Thirty-eight species of birds were analyzed. Linear regressions were used to derive equations and the species specific residuals were compared.

Encephalization indices were determined from the avian equation: log E=-0.860+log S 0.56; r=0.943. The slope of 0.56 (+0.105), which has been found by many other investigators (Portmann, Alauda, 1947) was confirmed. The slope is lower than the mammalian one (0.76), showing that for every unit increase in brain weight, birds gain more body weight. Classically Passeriformes, Strigiformes, Piciformes and Psittaciformes have larger brains for their body weights than do other birds (Ibid). Insufficent data precluded any analysis of Piciformes and Psittaciformes. Passerines (12 species) and owls (3 species) scaled similarly and had larger brains for their body weights than did the other birds (passerine + owl $t_{(32)}=4.04$, $p<.05$; passerine $t_{(27)}=2.97$, $p<.05$; owl $t_{(20)}=10.43$, $p<<.001$).

Adjusted encephalization indices (AEI) take into account O_2 turnover and are derived from the equation log E=-1.77 +0.828 log (SxBMR); r=0.918. When so adjusted, passerines and owls scale differently. Passerine AEI's cannot be distinguished from those of other birds ($t_{(29)}=0.115$, n.s.). Owls, on the other hand maintain a significant separation ($t_{(20)}=5.85$; $p<01$) indicating that at least two different systems for the delivery of glucose and oxygen to the avian brain exist. An adjustment of body weight for BMR thus accounts for differences between passerines and most other avian species. Owls, on the other hand, remain separated from the other birds, a situation analagous to the differentiation of primates from other terrestial mammals.

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253.14 **ALLOMETRY OF SOME MAJOR CNS DIVISIONS IN MICE, GERBILS, AND RATS: INTRASPECIFIC AND INTERSPECIFIC COMPARISONS.** **J. H. Fox* and W. Wilczynski. Dept. of Psychology, University of Texas at Austin, Austin, TX 78712.**

Heads and necks of CD-1 mice, Mongolian gerbils, and Sprague-Dawley rats were fixed in formalin and the brains and spinal cords removed. Spinal cord cross-sectional areas (SCAs) were determined through sectioning at the level of the 1st cervical vertibrae. Brains were weighed and then dissected into forebrains, cerebellums, and brain stems, and weights were taken of each of these divisions. Linear regression analyses were then performed on the relationships between logs of body weight and logs of the above five measures for each of the species; similar analyses were performed between species. The analyses were used to determine exponents for the equation, $E=kP^a$, where E is a brain (or spinal cord) measure, and P is body weight.

Intraspecific comparisons yielded no significant relationships between body weight and any measure. Because the SCA should differ, within a species, according to numbers of spinal axons, it is suggested that no differences in total somatic innervation are systematic with body weight. Differences occur interspecifically, however. Following are the resulting exponents and related information (all tests 1-tail, 1 df): brain weight, a=.540 (r=.99936, p<.025); forebrain weight, a=.550 (r=.99917, p<.025); cerebellum weight, a=.562 (r=.99935, p<.025); brain stem weight, a=.544 (r=.99978, p<.01); SCA, a=.375 (r=.98772, p<.05). The lower exponent (relative to 2/3) for brain weight in this study (as well as in other studies examining closely related species or individuals within single, indeterminantly growing species) cannot be explained by any differential scaling of major brain regions (which all vary with about the .55 power of body weight). The exponent for the SCA may be lower than the other exponents because it relates to an area, not a volume; indeed, when the areas are raised to the 3/2 power (to generate "volumes"), the exponent increases to .562. Whether the area or "volume" measure is preferable is uncertain. The area measure, although it involves confusing units, provides an estimate, when corrected for average fiber diameter, of the total number of somatic afferents and efferents, which is of relevance to theories relating brain size to somatic information processing. The exponent for the SCA measure is far from 2/3, suggesting that body surface area does not predict the total information passing between the brain and the periphery. Future work will determine allometric functions of major CNS areas across more distantly related mammals, including humans. It is hoped that data from SCAs will provide the best basis from which to estimate somatic influences on brain allometry. Thanks are due to D. Thiessen for lab space and to B. Cocke, C. Smith, T. Schallert, W. Dillon, and J. Letchworth for animals.

253.15 A PECULIAR ARTERIO-VENOUS LEPTOMENINGEAL BUNDLE IN THE GUINEA PIG. B. Azzarelli. Indiana University, Department of Pathology, Indianapolis, IN 46223

Arteries and veins often run in close approximation sharing a common connective tissue sheath. One exception to this generalization is seen in the brain where arteries and veins run separately. Classically, the blood vessels down to the arterial and venous level are formed by three coats: intima, media and adventitia. Leptomeningeal vessels are further reinforced by a monolayer of pial cells.

We have discovered in the guinea pig, superior to the corpus callosum, running from the level of the rostrum to the splenium, a group of richly innervated blood vessels (an artery and several veins) enclosed in a common leptomeningeal sheath. The artery arises at the point of confluence of both anterior cerebral arteries: the veins drain into the transverse sinus. The epithelial nature of the sheath is evident by the close apposition of cell membranes, the presence of junctional devices, and the existence of basal lamina. The ultrastructural features of this epithelium are similar to those of the arachnoid-dural membranes. Presently, we can only speculate on the significance of this vascular bundle. In some cases a close association between arteries and veins accounts for a countercurrent exchange of heat or ions from the incoming to the outgoing vessels. The presence of these vessels apparently "isolated" within a leptomeningeal subcompartment may provide a suitable model to study vascular - extravascular - cerebrospinal fluid substance exchange.

253.16 THE MORPHOLOGICAL AND THE FUNCTIONAL PATTERN OF THE BRAIN STEM. R. Nieuwenhuys*. (SPON: P.G.M. Luiten). Dept. of Anatomy, Univ. of Nijmegen, Nijmegen, The Netherlands.

According to the classical doctrine, developed by His, Gaskell, Herrick and Johnston, the brain stem consists of four longitudinally arranged areas, each of which can be functionally characterized. Thus, a somatomotor area ventralis, a visceromotor area intermedioventralis, a viscerosensory area intermediodorsalis and a somatosensory area dorsalis can be distinguished. The boundaries between these zones are generally marked by ventricular grooves, i.e. from medial to lateral the sulcus intermedius ventralis (siv), sulcus limitans (sl) and sulcus intermedius dorsalis (sid). In order to test the validity of this doctrine a procedure has been developed by us, making it possible to survey in one figure the neuronal structures and their relationship to the ventricular grooves in this region. Essentially this procedure involves two steps: (1) the cell masses are projected upon the ventricular surface, and (2) the ventricular surface is flattened out, i.e. subjected to a one-to-one topological transformation. In our laboratory such topological analyses were carried out on the brain stems of 16 different species, ranging from the lamprey to the turtle. These analyses yielded the following results. In the rhombencephalon the gray matter is arranged in four longitudinal areas and in many places the siv, sl and sid mark the boundaries between these morphological entities. These areas coincide largely, but not entirely with the functional columns of previous authors. The most obvious incongruity is that the area intermediodorsalis contains, in addition to two viscerosensory nuclei, a number of evident somatosensory cell masses. The four longitudinal zones cannot be distinguished in the mesencephalon nor can the sl be recognized there. Functionally, however, the medial part of the tegmentum mesencephali may be considered the rostral extreme of the somatomotor column, whereas the remainder of the midbrain contains a number of somatosensory centers.

Apart from presenting an overview of the structural relations in a given brain stem, the topological charts produced have also appeared to be useful for showing the pattern of connectivity of the various centers, as well as for mapping the results of experimental work with retrograde tracers.

CHEMICAL SENSORY SYSTEMS II

254.1 AXONAL PROJECTIONS AND CONDUCTION PROPERTIES OF OLFACTORY PEDUNCLE NEURONS IN THE SOUTH AMERICAN ARMADILLO (CHAETOPHRACTUS VELLEROSUS). H. Ferreyra and A. Cinelli*. Instituto de Investigación Médica M. y M. Ferreyra. 5000 Córdoba, Argentina.

Axonal projections and conduction properties of olfactory peduncle (OP) and visual callosal neurons have been investigated in endothermic mammals (rat, rabbit, monkey) with core temperatures of 36-38°C. We have sought to extend these observations to a primitive edentate, the armadillo, wich normally displays a low core temperature of 32-34°C.

Extracellular antidromic single action potentials were recorded from 67 neurons throughout the OP region following electrical stimulation of the ipsilateral (N=41) and contralateral (N=26) olfactory bulb (IOB;COB) at core temperatures of 28-32°C in 20 armadillos under Urethane (1 g/kg) anesthesia. Absolute refractory period (ARP), axonal conduction velocity (CV), super and subnormal periods (SPN;SBN) were evaluated. The ARP and the CV were negatively correlated ($r= -53$; $p < 0.001$). An early SPN and late SBN period of increased and decreased CV and excitability, respectively, was found in 82 % (SPN) and in 58 % (SBN) of neurons tested for variations in antidromic latency and threshold to the second of two identical volleys delivered to the OB at C/T intervals of 1-2000 msec. Latency variations ranged between 0.5 to 12 % of antidromic control latency. Both the SPN and SBN period were negatively correlated to the CV ($r= -.55$, $p<0.001$) indicating that larger axons have shorter SPN and SBN periods than thinner ones. Both the magnitude and the duration of the SPN and SBN periods were positively correlated to the CV ($r= .55$, $p<0.001$ and $r= .58$, $p<0.002$, respectively). The duration but not the magnitude of the SPN and SBN period were also correlated ($r= 0.79$, $p<0.001$). During the SBN period, the antidromic latency of 15 neurons increased in a gradual additive manner, reaching an apparent asymptotic value after 60-128 stimuli. These results indicate that axonal conduction properties of OP neurons in an ancient eutherian mammal are not invariant in the temporal domain but rather change depending on the history of previous activity in the axon.

Supported by CONICOR and CONICET.

254.2 ODOR RESPONSES OF ANTIDROMICALLY-IDENTIFIED OLFACTORY BULB OUTPUT NEURONS IN THE RAT. Theresa A. Harrison and John W. Scott. Dept. of Anatomy, Emory Univ., Atlanta, GA 30322

The two major morphological types of olfactory bulb (OB) output neurons, mitral and tufted cells, can be distinguished during unit recording by the different projection sites from which they can be antidromically activated. Recent work from this laboratory (Schneider & Scott, J. Neurophys., 50, 1983) showed that antidromically identified tufted cells differed from mitral cells in being more easily activated by electrical stimulation of olfactory nerve, and in responding more often with multiple spikes. These data support the hypothesis that mitral and tufted cells function differently in the processing of primary olfactory information. In the present study, this hypothesis was further tested by examining the responses of identified OB output neurons to different concentrations and qualities of odors, in Nembutal-anesthetized rats.

As in the previous study, OB units were classified as: OT units, if antidromically activated from olfactory tubercle but not posterior piriform cortex; pPC-OT units, activated from both sites; and LOT units, activated only from the rostral lateral olfactory tract. Odor stimuli were delivered by using an infusion pump to push air at variable rates through a chamber containing odorant-saturated filter paper and into a clean air line ending in a chamber over the rat's nose. This system permitted rapid switching between 6 different concentrations and any of 32 odors. The concentration of odor delivered was established by prior calibration of each odor source, using a gas sampling valve-equipped gas chromatograph.

Odor responses of 31 OT and LOT units, and 16 pPC-OT units have been studied. Initial results indicate that OT and LOT units are more easily activated by odors than are pPC-OT units. Sixty percent of the pPC-OT units were unresponsive to any odor tested, even at highest concentrations. In contrast, 85% of the OT and LOT units responded to more than one odor, and some to all odors tested, down to the lowest concentrations. This is consistent with the previous finding of lower thresholds and greater responsiveness of presumed tufted cells to electrical stimulation of the olfactory nerve. Temporal patterns, direction and amplitude of odor responses varied under these experimental conditions; a sample adequate for evaluating systematic differences between cell types in these respects is currently being collected. Supported by Public Health Service Grant #NS 12400.

254.3 TASTE REACTIVITY AFTER BILATERAL GUSTATORY NERVE SECTION
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Attempts to assess the effects of gustatory deafferentation on ingestive behavior have used both short and long term consumption tests (e.g. Jacquin, Beh. Neuros. '83; Pfaffmann, JCPP '52). Recent studies have demonstrated that gustatory sensitivity can be assessed using intraoral stimulation and scoring the resultant oro-facial movements videographically (Grill & Norgren, Brain Res.'78) or electromyographically (Travers & Norgren, Neuros. Abst. '83). We have begun to test the effects of selective, bilateral gustatory nerve cuts on the production of oro-facial movements to intraoral sapid stimulation. Stimulation and recording techniques are essentially the same as described in Travers & Norgren ('83). Adult rats fitted with intraoral cannulas and chronic EMG electrodes from up to 4 muscles were tested with 5 concentrations of sucrose, NaCl, QHCl, and water. Following collection of baseline data, one group of rats was anesthetized and the chorda tympani nerves (CT) cut bilaterally intra-aurally. Following recovery, animals were retested electromyographically. Subsequently, the rats were tested independently in a second laboratory using the same stimulation techniques and responses videotaped for a frame by frame analysis of taste reactivity components. A second group of animals received bilateral IX nerve cuts at the time of the EMG electode and intraoral cannula implants but were tested with identical procedures as the CT group. A third group of animals received no nerve cuts. A normal response to suprathreshold QHCl consists of several licks, a series of gapes, a brief pause (2-3 sec) and a second series of gapes. Following CT nerve cuts rats showed an increase in the number of licks preceding the 1st gape response (longer latency) as well as some indication of a heightened response threshold. Rats with IXth nerve cuts exhibited a reduced latency to the first gape at low concentrations but no threshold change for the first series of gapes. The second gape bout, however, was absent. These preliminary observations suggest that neural signals in either the CT or IXth nerves can initiate rejection but that both nerves are required for an electromyographically normal response.
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254.4 TASTE RESPONSES IN THE NUCLEUS TRACTUS SOLITARIUS OF THE BEHAVING MONKEY.
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Recent autoradiographic studies have precisely delimited the gustatory relays in monkey brainstem and so made them more accessible to the electrophysiologist. We recorded multiunit and single neuron responses from the nucleus tractus solitatius (NTS) of two cynomolgus monkeys (Macaca fascicularis). Under barbiturate anesthesia each was fitted with a stainless steel headmount which accommodated a micropositioner for subsequent daily recording. Electrodes were glass coated tungsten plated with platinum black and gold chloride (tip size, 2 x 4 microns). Stimuli were 1.0mM - 3.0M NaCl, 1.0mM - 3.0M glucose, 0.01 - 30.0mM HCl, 0.001 - 10.0mM quinine HCl and 20% blackcurrant juice.

The monkey NTS is a nearly cylindrical structure with its main axis oriented about 30° to the anterolateral. It ranges from 3.5 - 5.5mm lateral to the midline and from 0.5 - 3.0mm posterior to the auditory meatus (ear bar zero). Multiunit intensity-response functions in NTS were comparable to those reported psychophysically. However the magnitude of response evoked by each stimulus was dependent upon location within NTS. Sensitivity to HCl was greatest toward the posterior of the gustatory region while responses to sweet stimuli, NaCl and even quinine were more robust anteriorly. This crude chemotopia provides one possible mechanism for quality coding. Time courses were also distinctive among the basic qualities: HCl evoked the largest phasic peak, and glucose the most prolonged activity.

We isolated 43 neurons from throughout NTS. Spontaneous rates were near 1.2/sec. Evoked responses were a function of location, with sensitivity generally paralleling the multiunit activity of that subarea. Discharge rates rarely exceeded 100spikes/5 sec, though it is likely that more responsive neurons were bypassed because better isolation is required to follow their activity. Cells were broadly sensitive to the basic stimuli, though less so than in the rat NTS. Finally we tested the responsiveness of neurons to 1.0M glucose before, during and following satiating glucose loads to determine whether motivation or hedonic appeal affected NTS sensitivity. We saw no apparent changes in responsiveness.

254.5 PERIPHERAL AND CENTRAL EVENTS CONTRIBUTE TO MIXTURE SUPPRESSION IN THE OLFACTORY PATHWAY.
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Marked suppression between or among the components of stimulus mixtures is common in both olfaction and taste. The role of peripheral and central events in this phenomenon is a paramount issue in understanding the perception of complex odors and tastes. We are studying mixture suppression in a relatively simple olfactory system, that of the spiny lobster, which shares basic organizational features with the vertebrate olfactory pathway. Using activity evoked in brain-output interneurons as an assay, we have identified the components of a food odor that are stimulatory and/or interact with other components in the mixture. Based on a non-additive model, 12 components were stimulatory. Of these, two were also synergistic and one was suppressive. Three other components were non-stimulatory, but suppressive. The remaining 16 of the 31 components analyzed did not contribute to the mixture. Confirmatory experiments failed to support any overall synergistic effect, in contrast to supporting a strong overall suppressive effect. Both peripheral and central events appear to contribute to the observed mixture suppression. Single unit recordings from receptor cells identified a class of taurine-excited cells whose response to taurine is mostly or entirely suppressed by at least 6 other amino acids in the mixture. At least two of the 6 suppressive amino acids appear to competitively inhibit the taurine response. That such receptors in fact contribute to the mixture suppression observed in brain-output interneurons is suggested by the finding that 75% of these interneurons show mixture suppression when stimulants and suppressants are presented co-spatially, yet fail to do so when the suppressants are applied co-temporally, but spatially separated from the stimulants. That the remaining 25% of these interneurons, however, continue to exhibit mixture suppression under this "split-nose" paradigm indicates that the suppression is also generated in part within the CNS. The nature of the suppression generated within the CNS is presently unknown.
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254.6 CENTRAL ORGANIZATION OF AFFERENT FIBERS OF THE POSTERIOR TONGUE IN THE HAMSTER.
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Taste and touch sensibilities for the posterior tongue are mediated by the glossopharyngeal nerve. The central projections of its lingual branch (L IX) were visualized with horseradish peroxidase.

Afferent fibers enter the ventrolateral medulla 350 um caudal to the cochlear nucleus and course rostrally and medially to enter the solitary tract at the level of the cochlear nucleus. En route to the solitary tract, some entering fibers bifurcate and send descending branches through the dorsal spinal trigeminal tract. These fibers extend far caudally and terminate densely in a marginal lamina of the spinal trigeminal nucleus (pars caudalis) and in deeper, restricted terminal fields. Fibers descending in the trigeminal tract also send collaterals medially to the solitary nucleus. Endings distribute to its rostral and intermediate subdivisions as well as to the rostral pole of its commissural nucleus. Similarly, labeled afferents descending in the solitary tract terminate at all rostrocaudal levels of the ipsilateral solitary nucleus. Minor projections to the reticular formation, area postrema, lateral cuneate nucleus, and to the contralateral commissural nucleus were noted. Terminal label is heaviest at intermediate levels of the solitary nucleus but becomes sparse rostrally and caudally. At rostral levels, terminal label covers the dorsal one-half of the nucleus but shifts to become concentrated ventrolaterally at more caudal levels.

The afferent projections reported here resemble those of the same glossopharyngeal branch in the rat (Hamilton, R.B. and Norgren, R., J. Comp. Neurol., 220: 378, 1984) and are compared to projections of anterior tongue afferents in the hamster (Whitehead, M.C. and Frank, M.E., J. Comp. Neurol., 222: 560, 1983). L IX and chorda tympani projections are largely coextensive in the solitary nucleus, except rostrally. These projections are also overlapped by afferents of the lingual (trigeminal) nerve in the lateral part of the intermediate solitary nucleus. Thus, this area receives converging inputs from the anterior and posterior tongue via 3 cranial nerves mediating at least two sensory modalities.
(Supported by NS16993 and the CT. Research Foundation).

254.7 MITRAL AND TUFTED CELLS OF THE RAT OLFACTORY BULB VISUALIZED BY INJECTION OF THE PHAGEOLUS VULGARIS LEUCOAGLUTTININ LECTIN (PHA-L). J.W. Scott, J. Pemberton* and E.C. Rainer*. Dept. of Anatomy, Emory Univ., Atlanta, GA 30322.

The PHA-L lectin is useful for tracing anterograde connections using survival times of several days (Gerfen and Sawchenko Brain Res. 290:219, 1984). We used small, iontophoretic injections of PHA-L into the external plexiform layer (EPL) of the rat olfactory bulb (OB) to trace the projections from mitral and tufted cells. Survival times varied from 4 to 10 days. Tissue was cut in the sagittal plane at 80 micrometers, defatted with ethanol and processed immunohistochemically using an avidin-biotin-peroxidase procedure with a cobalt intensified DAB chromogen. Each injection labelled up to 10-15 mitral and tufted cells and a varying number of granule cells. Cells were reconstructed through serial sections. The ultimate goal is to describe the distribution of axons of the projection cells, but this preliminary report deals primarily with intrabulbar and anterior olfactory nucleus (AON) connections.

Since PHA-L survives in the neuron for a longer period, it is important to observe whether the results agree with those seen with horseradish peroxidase injections (Orona et al., JCN 217:227, 1983; Orona et al., JCN, 1984). As in those studies, all labelled neurons have processes that extend into the injection site. The lengths and distributions of mitral and tufted cell basal dendrites agree closely with our earlier work. We confirmed the division of mitral cells into two major types depending on the position and lengths of basal dendrites. Axon collaterals within the internal plexiform layer of the OB come only from tufted cells and mitral cells with superficial basal dendrites (type II). While the basal dendrites of granule cells are more intensely filled after PHA-L injection, the conclusion of differential innervation of the EPL by superficial and deep granule cells is still supported.

The PHA-L injections enabled us to follow projection cell axons for several mm. depending upon the survival time. Most projection cells have several axon branches and all of the mitral cells we have studied project into the posterior part of the lateral olfactory tract or onto the posterior piriform cortex. We have confirmed that some but not all mitral cells have axon collaterals that terminate in the AON superficial to the pars externa. Initial results indicate that type I mitral cells, particularly those with larger axons, are less likely to have such collaterals. Supported by Grant #BNS-8102175 from NSF.

254.8 AFFERENT PROJECTIONS OF THE SUPERIOR LARYNGEAL NERVE TO THE MEDULLA OF THE LAMB. R. D. Sweazey and R. M. Bradley. Dept. Oral Biology, Univ. of Michigan, Sch. of Dentistry, Ann Arbor, MI 48109.

To determine the central projections of afferents innervating taste buds on the epiglottis, horseradish peroxidase (Sigma VI) was applied to the cut end of the superior laryngeal nerve (SLN) in 10 lambs aged 30 to 60 days. After 72 hours transverse sections of the brainstem were processed using the tetramethyl benzadine method.

Afferent fibers of the SLN entered the ipsilateral brainstem through a series of vagal rootlets, from the level of the rostral inferior olive caudal to the level of the dorsal motor nucleus of the vagus (DMV). A small number of fibers joined the dorsal spinal trigeminal tract while the majority of fibers joined and ran caudally in the solitary tract (ST). Labeled terminals in the nucleus of the solitary tract (NST) extended from rostral levels of the DMV caudally to the commissural nucleus. At the level of the DMV the majority of the terminations were located in the medial NST. Further caudally, at the level of the area postrema, SLN terminals were located primarily around discrete bundles of ST fibers; a few terminals were located in the medial and ventrolateral NST. At the level of the commissural nucleus terminals were located exclusively in the lateral aspects of the nucleus. A few terminals were located in the brain contralateral to the labeled SLN. Finally, terminals also were located in the dorsal-lateral portions of the caudal spinal trigeminal nucleus.

The central projection of the SLN is quite extensive. We have shown using electrophysiological recordings that the chemosensitive fibers terminate in a limited part of this projection. Cells responsive to chemical stimulation of the epiglottis were located in the NST at the rostral regions of SLN terminations. The caudal most extent of chemical responsiveness was located 1 mm rostral to obex. The majority of these cells were located in the ventrolateral NST or just medial to the ST, at a depth of 2-3 mm. These second order chemosensitive cells responded to an array of chemicals similar to those previously described for single SLN fibers (Bradley, R.M., et al., Brain Res., 276:81, 1983).

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254.9 TERMINAL NERVE CENTRAL PROJECTIONS. C.R. Wirsig. Dept. of Neuroscience, Univ. of Florida, Gainesville, FL 32610.

The terminal nerve (NT) has been identified as a unique ganglionated nerve of the nasal cavity, separate from the olfactory, vomeronasal and trigeminal systems in vertebrates. Because its bipolar cell bodies are dispersed along its course, the direction of their axonal projections has been difficult to determine. Recently, a subpopulation of TN neurons containing luteinizing hormone-releasing hormone-like immunoreactivity (LHRH-ir) has been demonstrated in several species. In fish, retrograde tracers have demonstrated that some of these neurons project centripetally into the forebrain and into the retina (Demski, L. & Northcutt, G. 1983 Science 220:435). In the hamster, most LHRH-ir processes of peripheral and central TN neurons have a centripetal orientation. The fibers appear to be directed mainly toward the accessory olfactory bulb (AOB) and medial septum. Thus, to verify the direction of TN fibers, I examined the projections of the transected and non-transected TN in the Golden hamster. A coronal cut separated the olfactory bulb from the forebrain. After perfusion with Zamboni's fixative, standard immunocytochemical procedures were used to identify LHRH-ir neurons. Terminal nerve LHRH-ir cell bodies are bipolar or pseudo-unipolar and most of these that accompany both peripheral and intracerebral portions of the nerve possess varicose processes on their proximal side, i.e. directed centripetally. A small intracerebral ganglion, however, was observed that sends its axons in a centrifugal direction, toward the root and main ganglion of the TN. Whether these axons exit the brain or end in the main ganglion could not be established. Forebrain lesions caused an accumulation of LHRH-ir in fibers of the TN on the distal side of the cut, indicating a peripheral site of origin for these fibers. Since forebrain cuts did not reduce the staining of fibers travelling to the AOB, it appears that this projection originates in the TN, and not from a central location as previously hypothesized.

The AOB is involved in reproductive behavior in rodents, especially in the hamster. Exposing mice or voles to conspecific pheromones has been shown to increase AOB LHRH and blood LH (Dluzen, D. & Ramirez, V. 1983 Horm. Behav. 17:139). Since the source of AOB LHRH is apparently the TN, the TN may sensitize the AOB to specific incoming sensory signals. Thus the multiple projections of the TN to the AOB and to central nuclei may act to coordinate hormonal and behavioral events produced by pheromones. Supported by NS 13516 to C.M. Leonard and MH 15737-05 and Sigma Xi Grant to C.R. Wirsig.

254.10 OLFACTORY RECEPTOR NEURONS ARE A PERIPHERAL CONDUIT FOR ACCESS OF FOREIGN SUBSTANCES TO THE CENTRAL NERVOUS SYSTEM. H. Baker. Lab. of Neurobiology, Cornell Univ. Med. Coll, New York, NY 10021.

Olfactory receptor neurons located in the nasal epithelium are contacted continuously by molecules contained in inspired air. These cells, whose axons innervate the main olfactory bulb (MOB) might be an avenue by which foreign substances could reach the central nervous system. To investigate this hypothesis, the lectin, wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) was utilized since this protein, following intraocular injection, is transneuronally transported from retinal ganglion cell afferents throughout the visual system labelling cells in colliculus, geniculate and pretectum, as well as the cortex. WGA-HRP (0.1%, 50-75 ul) was instilled unilaterally into the nares of rats. After survival periods of 2-6 days, HRP was visualized by very sensitive TMB histochemistry. Labelling in the MOB was almost exclusively unilateral but varied from uniform, i.e., contained within all olfactory receptor afferents and thus all glomeruli, to assymetric with only laterally placed glomeruli containing label. In two animals the central target of the vomeronasal organ, the accessory olfactory bulb, was predominantly labelled. In all animals transneuronal transport of lectin was observed. In MOB heavy label was found in periglomerular, tufted, mitral and some internal granule cells. Following a 4-6 day survival, retrogradely labelled neurons were visualized in several regions of the preoptic area known to be cholinergic and to project to the MOB, including the vertical and horizontal limbs of the diagonal band, magnocellular preoptic area and with occasional cells in substantia innominata. Terminals, probably representing axonal projections of mitral cells, but not retrograde cell labelling, were visualized in olfactory tubercle, piriform cortex and surrounding the lateral olfactory tract. Since these areas are also thought to contain neurons projecting to the MOB, retrograde cell label may depend on the distribution of centrifugal afferent terminals within the MOB. Axons from regions not displaying cell labelling terminate predominantly in the internal granule cell layer, whereas, those from regions which do contain retrograde cell labelling, have heavy terminal fields in the glomerular layer of the MOB. Using comparable procedures terminal labelling was also observed in the forebrain of the cat. These data demonstrate that some molecules entering olfactory receptor cells can move transneuronally through the olfactory bulb to reach neurons in the area of the preoptic region known as the basal forebrain. It is interesting to note that cholinergic neurons in basal forebrain are thought to be involved in Alzheimer's Disease.

254.11 TOPOGRAPHIC REPRESENTATION AND ULTRASTRUCTURE OF GUSTATORY AFFERENTS IN THE GOLDFISH, CARASSIUS AURATUS. Y. Morita and T. E. Finger, Dept. of Anatomy, Univ. Colo. Med. Sch., Denver, CO 80262.

The vagus nerve of the goldfish provides sensory innervation to the oropharyngeal cavity including the gill arches and the palatal organ, a fleshy organ attached to the roof of the mouth. These oropharyngeal structures are equipped with a large complement of taste buds. The gustatory fibers of the vagus nerve terminate centrally in the vagal lobe, an enlargement of the special visceral sensory column of the medulla. The vagal lobe is clearly laminated and contains both sensory and motor elements. In the present study, we utilized horseradish peroxidase to examine the pattern of central termination of the different peripheral branches of the vagus nerve.

The vagus nerve as a whole terminates in sensory layers 2, 4, 6, and 9 of the vagal lobe (terminology of Morita et al., '80). The palatal organ nerve terminates in layers 6 and 9 while the gill arch nerves terminate in layers 2, 4 and 9. The gill arch nerve projection can be further dissociated into that portion arising from the gill raker nerves (layers 4 and 9), and that portion from the gill filament nerves (layer 2, and layers 4 and 9 in the ventral one-third of the lobe).

Differential HRP injections into the various branches of the vagus nerve also reveal a topographically organized representation of the oropharyngeal cavity in the vagal lobe. The anterior parts of the palatal organ and first (anterior) gill arch are represented most anteriorly in the lobe. The area of the oro-esophageal junction and fifth gill arch are represented most posteriorly. Ventromedial portions of the palatal organ and gill arches are represented ventrally in the vagal lobe while dorsolateral portions of the oropharyngeal cavity are represented dorsally in the lobe. The representations of the different gill arches overlap slightly with one another. Thus, the overall organization is such that the two opposing epithelial surfaces from one point of the oral cavity are represented in different layers of a single locus in the vagal lobe.

The laminar segregation of gustatory nerve fibers arising from two different epithelial surfaces allows us to compare the ultrastructure of gustatory terminals from two different sources. Following injections of HRP into the palatal organ, gill filament and gill raker nerves, EM analysis reveals, a) synaptic contacts are present in layers 2, 4, 6, and 9; b) the presynaptic boutons of each nerve contain clear, round synaptic vesicles regardless of layer of termination; and c) in many synaptic contacts in layer 4 the postsynaptic dendritic element contains pleomorphic vesicles.

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254.12 STRAIN DIFFERENCES IN SALT PREFERENCE OF RATS. E.E.Midkiff* and I.L.Bernstein (SPON: D.F. Farrell).Dept. Psychol. Univ. of Washington, Seattle, WA 98195.

In general, genetic contributions to taste sensitivity and preference have received little experimental attention. The determinants of salt intake and preference are of considerable interest, particularly because dietary salt has been implicated in the development of hypertension. The present study began an assessment of possible genetic contributions to salt (NaCl) intake and preference by examining the saline preference thresholds and "preference-aversion" functions of different strains of rats. Four strains were included; outbred Wistar and inbred Munich-Wistar, Buffalo and Fischer strains. Ten adult male rats of each strain were given continuous access to separate bottles containing deionized water and saline. Saline was presented in increasing concentrations from .06% to 1.9% for two days at each concentration, with bottles alternated daily to control for position preference. Saline intake was expressed both as a preference ratio (mls of saline divided by total fluid intake) and as intake adjusted for differences in body weight (mls/ 100g body weight). A significant strain difference was evident with Fischer rats differing markedly from the other strains. Wistar, Munich-Wistar and Buffalo rats showed preference-aversion functions similar to those which have frequently been reported for rats; a strong preference for saline over water at hypotonic concentrations (with a peak preference between .5 and .9%) declined sharply as concentration increased. In contrast, Fischer rats never demonstrated a preference for saline over water at any saline concentration. Further, they significantly preferred water to saline, beginning at .7% NaCl [$t(18)=2.77$; $p<.02$]; at this concentration animals of other strains are approaching maximum saline preference. Similar results were obtained when absolute saline intake adjusted for body weight was examined. The failure to demonstrate a saline preference at any saline concentration is strikingly different from the preference behavior which has typically been reported for the rat. Based on the responsiveness to other taste stimuli such as sucrose, citric acid and quinine, it appears that the difference between Fischer rats and other rat strains may be specific to salt stimuli.

254.13 ODOR FACILITATION OF LEARNED AVERSIONS TO COPULATORY BEHAVIOR IN MALE RATS. G. J. Lawrence and S. W. Kiefer, Department of Psychology, Kansas State University, Manhattan, KS 66506.

Male rat copulatory behavior can be modified or eliminated if the behavior is paired with gastrointestinal distress (Peters, Behav. Neurol., 1983, 97, 140-145). In the present study, the role of a neutral odor in copulatory aversions was examined.

In two experiments male rats were made ill with intragastric intubations of lithium chloride (2% body weight of a .15 M solution) following encounters with an estrous female over eight acquisition trials. In the first experiment illness was induced following an ejaculatory response during a 20 min pairing with the estrous female. In the second experiment illness was induced following an ejaculatory response or at the termination of the 20 min encounter, noncontingent on mating behavior. One group of males was trained with normal females (sex/ill) while a second experimental group was trained with a female whose anogenital region had been sprayed with a 2% aqueous solution of almond extract (odor-sex/ill). The copulatory behavior of these two groups was compared to appropriate control groups (sex/control, odor/control, illness/control).

Male rats made ill following exposure to an odorous female developed significant copulatory aversions in both experiments. On the eighth and last acquisition trial, 60% of males in Experiment 1 and 100% of males in Experiment 2 in the odor-sex/ill group refused to initiate any copulatory behavior. When ejaculation by the odor-sex/ill rats did occur during the early trials, latencies to mount, intromit, and ejaculate were significantly longer than control rats. Only weak aversions were noted in rats which were made ill after copulation with a normal female (sex/ill group). By the end of training, less than a third of the rats had developed copulatory aversions. Despite weak aversions, rats in the sex/ill groups did show significantly longer latencies than controls. A consistent finding in both experiments was that rats in the sex/ill and odor-sex/ill groups required significantly fewer intromissions to ejaculation than the control rats.

The results indicate that a novel almond odor significantly facilitated the acquisition of a learned copulatory aversion in male rats. Because the olfactory and vomeronasal systems have been shown to play an important role in mating behavior in rats, it may be that additional odor cues during mating behavior are easily associated with illness.

254.14 CANINE BEHAVIORAL RESPONSES TO OLFACTORY STIMULI. D.H. Krestel-Rickert* and F. Regnier* (Spon: C.A. Baile). Dept. of Biochem., Purdue Univ., W. Lafayette, IN. 47907.

Behavioral and physiological measures were recorded via video equipment and a telemetric device, in order to monitor male canine olfactory responses to odors. Studies were conducted 1) to examine responses to pure chemical odorants and "biologically significant" odors and 2) to examine relative effectiveness of four concentrations of 5 urine types. Summated response time values across 8 behaviors indicated that "biological" odors, specifically female urine, were the most potent. Overall behavioral means (trials=66) were as follows: estrous urine ($\bar{x}$=14.8 min), anestrous urine ($\bar{x}$=14.4 min), vaginal wash ($\bar{x}$=4.3 min), Amyl Acetate ($\bar{x}$=1.8 min), Methyl p-Hydroxybenzoate ($\bar{x}$=1.7 min), Butyric Acid ($\bar{x}$=1.4 min), Saline ($\bar{x}$=1.2 min), and water ($\bar{x}$=.7 min). Differences (ANOVA; $F=9.04$, $p<0.01$) existed between odors as measured by total response time with estrous urine being different from all other odors except anestrous urine. Differences between odorants for each of the behaviors also existed with sniff ($F=8.92$, $p<0.01$) directed primarily toward anestrous and estrous urine; urinate on the stimulus ($F=4.76$, $p<0.01$) directed toward estrous urine and head one foot near the stimulus ($F=3.74$, $p<0.01$) directed toward anestrous and estrous urine. In the second study urine concentrations used were undiluted, 8, 16, and 125-fold dilutions. Fresh male dog, fresh female estrous, aged estrous, aged anestrous, and fresh human urine were the urine types tested. ANOVA revealed that the eight behaviors could be categorized by significance of odor and/or concentration. The odor (O), concentration (C), and O x C factors were significant for sniff and lick ($F=11.4, 12.5$; 10.01, 6.00; 2.51, 3.19 respectively, $p<0.01$); categorized as chemoreceptive behaviors. Factors O and C were significant for salivate ($F=3.67$, 4.30) and urinate on the stimulus ($F=4.16$, 5.73, $p<0.01$). Only C was significant for urinate by ($F=7.12$, $p<0.01$) and was categorized with salivate and urinate on as chemoemittive behaviors. Only the odor factor was significant for paw at ($F=7.12$, $p<0.01$) and nudge ($F=3.13$, $p<0.05$); categorized as tropistic behaviors. Concentration effectiveness decreased logarithmically with decreasing concentration. Biologically significant odors, especially estrous female urine, were recognized as potent stimuli over chemical odors commonly used in olfactory studies. Also, certain behaviors are diagnostic of odorant and concentration effectiveness.

254.15 AMINE VAPORS AND A LIPOPHILIC ION CHANNEL LIGAND INHIBIT OLFACTORY TRANSDUCTION. Stephen P. Fracek, Jr., Bruce D. Winegar, and Rollie Schafer. Department of Biological Sciences, North Texas State University, Denton, TX 76203.

Certain amine vapors have two major effects on the olfactory mucosa of the frog: (1) the first exposure to the amine produces an immense electroolfactogram (EOG) response, which is often two to eight times greater than responses to other odorants, and (2) after the first exposure, EOG responses are inhibited.

Amines are unique among organic compounds in their extreme basicity. For example, diethylamine (DEA) is about 100 times more basic than ammonia. We tested a variety of amines and found that amines with a high vapor pressure and strong basicity were effective inhibitors of olfactory transduction. The role of basicity in inhibition was demonstrated by the use of structurally similar amines that differed in basicity, but which had approximately the same molecular weight, size, and vapor pressure. For example, comparisons of the inhibitory power of pairs of amines, such as pyrrole/pyrrolidine and pyridine/piperidine showed that the more basic saturated aliphatic compounds are more inhibitory than their less basic aromatic analogs. Similarly, when concentrations of amines with similar basicities were equalized, equal inhibitory power resulted (e.g. DEA and di-i-amylamine).

We believe that such amines must enter the olfactory neuron to inhibit it. The extreme basicity of the inhibitory amines given in sufficient concentration would elevate the extracellular pH so that a proportion of the amine molecules would remain in the neutral state and thus would be capable of penetrating the lipid membrane. Indeed, when the pH of the mucosa was lowered by the application of CO_2 (through the formation of carbonic acid), DEA was not inhibitory. Presumably the ionized form (conjugate acid) of DEA is barred from entering the cell.

It is likely that the inhibitory amines, once inside the cell, are converted to the ionized form through intracellular buffering. A working hypothesis is that inhibition occurs by interaction with ion channels associated with the transduction process, in a manner analogous to known "channel blocking" agents. In support of this idea we found that a lipophilic cation, tetraphenylphosphonium, a known channel blocker, produced inhibition. We conclude that neutral amine molecules enter the cell by diffusion across the membrane's lipid bilayer, become ionized within the cell, then inactivate ion channels from the inside of the cell.

254.16 A UNIQUE INTEGRAL MEMBRANE GLYCOPROTEIN OF FROG OLFACTORY CILIA: BIOCHEMISTRY AND IMMUNOFLUORESCENCE LOCALIZATION. Zehava Chen*, Dov Ophir* and Doron Lancet (Spon: A.Isseroff). Dept. of Membrane Research, The Weizmann Inst. of Science, Rehovot, Israel.

The glycoprotein gp95 is one of several glycosylated polypeptides which we have recently shown to be extractable from a preparation of isolated frog olfactory cilia by non ionic detergents (Chen and Lancet, Proc. Natl. Acad. Sci. 81 (7) [1984]). This relatively major ciliary glycoprotein has high reactivity with the lectin wheat germ agglutinin (WGA), while the other glycoproteins are recognized better by concanavalin A. Fluorescently labelled WGA stains the surface of isolated olfactory (but not respiratory) cilia, and labels the most superficial layer of intact olfactory epithelium. These findings are consistent with gp95 being a specific component of the external ciliary surface. We now have evidence that gp95 is unique among the ciliary glycoproteins also in being tightly associated with the lipid bilayer and in having a low isoelectric point, i.e. behaving as an integral membrane protein. In studies of protein biosynthesis in epithelial explants it appears to be replaced at a relatively high rate. The polypeptide gp95 thus appears to be the only ciliary protein which fulfils several important criteria for being olfactory receptor: high membrane concentration, tissue specificity and localization, surface disposition, lipid bilayer insertion, glycozylation and rapid turnover.

We have raised monoclonal antibodies against triton X-100 extract of the isolated olfactory cilia preparation. Several clones reacted against gp95 judged by immunoblotting. These antibodies yielded tissue immunofluorescence staining patterns similar to those of wheat germ agglutinin, and also specifically stained the surface of isolated olfactory cilia. The antibodies are now being used together with lectins to probe the possible role of gp95 in olfactory function. This is done through topical applications to the epithelial surface while recording sumated electrophysiological responses to odorants in vivo. In parallel, we study the polypeptide heterogeneity of gp95, in view of the hypothesis that olfactory receptors constitute a family of proteins with similar overall structure, but with slight variations in amino acid sequence for different odorant specificities.

254.17 ELECTROPHYSIOLOGICAL STUDIES OF CHEMORECEPTION IN PARAMECIUM. J. Van Houten, R. Preston*, S. Schulz.* Dept. of Zoology, Univ. of Vermont, Burlington, VT 05405

Paramecia detect chemicals in solution around them. In the case of potassium folate (K_2-folate), the chemoattractant binds to specific sites on the cell body membrane (Schulz, S. et al., J. Cell Bio. 97: 479a, 1983). This binding is somehow transduced into a hyperpolarization of the cell relative to the membrane potential (Vm) in twice the molar concentration of potassium chloride (2x KCl) (Van Houten, J. Science. 204: 1100, 1979). Other attractants such as acetate (OAc) and ammonium (NH_4^+) also hyperpolarize the cells relative to control salts, and the mechanisms by which cells hyperpolarize in attractants is being examined using standard electrophysiological techniques.

Deciliated cells respond with normal hyperpolarizations in OAc, NH_4^+, and K_2-folate, indicating that components necessary for the change in Vm in these attractants are on the cell body and are not exclusively on the cilia. Local pressure perfusion of folate onto deciliated cells during recording demonstrated a gradient of response of the cell from anterior decreasing to no measurable response at the posterior. These responses are not due to mechanoreceptor stimulus by the perfusion process. Fluorescently conjugated folate selectively stains normal cells in contrast to folate chemoresponse mutants, which cannot be not distinguished from background autofluorescence. This dye will be helpful in detection of any localization of folate binding sites on the cell bodies that may parallel localization of the Vm response.

Concentration studies indicate that cells respond with changes in Vm to changes in solution from K_2-folate to 2x KCl (and vice versa) over the entire concentration range tested (20 uM to 10 mM). The response curve is sigmoidal in shape, maximal at 5 mM and minimal by 50 uM. Ionic strength effects were examined in concentration studies holding K fixed at 5 mM while increasingly substituting folate for 2x Cl. The cells responded over the entire range tested (50 uM to 2 mM) but the curve was shifted to slightly higher concentration.

Permeability (P) studies in progress indicate no change in P_K for cells in folate or OAc relative to Cl, nor differences between P_{folate}, P_{OAc}, and P_{Cl}. Cells in OAc appear to have an increased P_{Ca} and P_{Na}. The role of these P changes in hyperpolarization is being investigated. (Supported by NIH GM29045 and NSF BNS12176.)

254.18 MIXTURE SUPPRESSION IN PRIMARY OLFACTORY RECEPTOR CELLS IN THE LOBSTER. B.R. Johnson*and J. Atema (SPON: C. Scheffey). Boston Univ. Marine Prog., Mar. Biol. Lab., Woods Hole, MA, 02543

The lateral filament of the lobster's antennules functions as an olfactory organ (Reeder, P. and Ache, B.W., Anim. Behav., 28:831, 1980 and Devine, D.V. and Atema, J., Biol. Bull., 163:144, 1982). Antennular hydroxy-proline (OH-Pro) and taurine (Tau) receptors are very narrowly tuned (Johnson, B.R. and Atema, J., Neurosci. Letts. 41:145, 1983) and show a suppressed response when a mixture of the following 15 compounds in equimolar concentrations is tested: Tau, OH-Pro, glutamate, ammonium chloride, arginine, sucrose, ethanol, alanine, lysine, betaine, aspartate, glycine, leucine, glutamine and proline. In the present study we determined the effect of the mixture on the response range of the OH-Pro and Tau receptors and identified the suppressive components.

We identified electrophysiologically single chemoreceptor cells responding to OH-Pro or Tau with an applied concentration of 10^{-4}M. Concentrations were diluted 40 fold after introduction into the test chamber. Single OH-Pro or Tau cells were then tested with ascending concentrations of 10^{-6}-10^{-3}M OH-Pro or Tau or the complete mixture. We determined the suppressive components by testing single OH-Pro or Tau cells with 10^{-4}M OH-Pro or Tau alone and with binary combinations of OH-Pro or Tau plus one of the mixture components at 10^{-4}M. Dose-response curves showed the OH-Pro cells were suppressed by the mixture across their entire response range, applied concentrations of 10^{-5}-10^{-3}M. In contrast, Tau cells were suppressed only at the highest applied concentrations, 10^{-4} and 10^{-3}M. The parallel shift of the dose-response curves for both the OH-Pro and Tau cells when tested with the mixture suggests a competitive inhibition of OH-Pro or Tau by mixture components. For each OH-Pro or Tau cell there were always several suppressive components within the mixture and these varied from cell to cell. The most effective suppressants for the OH-Pro cells were glutamine, arginine and lysine; the most effective for the Tau cells were ammonium chloride, arginine and OH-Pro.

Since natural stimuli occur in mixtures, the presence of other compounds may affect a receptor's response to its best stimulus and thus the animal's perception of particular stimuli. The primary receptors of crustacea provide a model system for physiological studies of mixture interactions at the receptor level.

Supported by Whitehall Foundation and NSF (BNS-8210434).

254.19 CHEMICAL SIGNALS AGAINST NOISY BACKGROUNDS. Jelle Atema, Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.

The normal noise background for marine animal chemoreception contains free amino acid concentrations in the pico- to nanomolar range; ammonia occurs in micromolar quantities. Amino acids are potent chemical stimuli for many organisms, from bacteria to fish. These stimuli must be discriminated against the chemical noise of the environment. Narrow tuning of primary receptors may be a mechanism to facilitate signal detection.

Lobsters have prominent populations of receptor cells which are narrowly tuned for single amino acids and ammonia. Such cells are found both in smell and in taste organs. One might expect, therefore, that elevating the normal background with one amino acid should raise the detection threshold of this compound to the new level, but should not interfere with the reception of another.

The lobster's antennular flicking rate was used to obtain single compound dose-response curves from 10^{-12}M to 10^{-3}M in single log steps. Stimuli were injected into sea water background flow. Indeed, in backgrounds elevated for just one compound, detection thresholds for that compound shifted up to the new background level; however, the entire dose-response curves also dropped indicating that even at high stimulus concentrations responses were suppressed (auto-adaptation). Contrary to expectation, similar suppression of entire dose-response functions was seen in cross adaptation experiments. In addition, in elevated backgrounds, lobsters responded to both higher and lower stimulus concentrations indicating that sudden temporary dilution of only one amino acid in the whole background mixture cannot only be detected but can constitute a behaviorally significant stimulus.

The results must be seen in light of mixture suppression of primary receptor cells (Johnson and Atema, Neurosci. Abstr., 1984).

Supported by Whitehall Foundation and NSF (BNS-82104434).

254.20 FUNCTIONAL ANATOMY AND PHYSIOLOGY OF MALE-SPECIFIC PHEROMONE-PROCESSING INTERNEURONS IN THE BRAIN OF *MANDUCA SEXTA*. T.A. Christensen and J.G. Hildebrand. Dept. of Biol. Sci., Columbia University, New York, NY 10027.

The hawkmoth *Manduca sexta* exhibits a striking sexual dimorphism such that only the male moths detect the pheromone released by sexually receptive females. A male-specific olfactory subsystem, comprising specialized elements throughout the olfactory pathway, is responsible for processing sensory information about the female pheromone. We study CNS neurons in this subsystem to reveal general olfactory mechanisms as well as details of the male's sensitivity to pheromone. On each antenna there are ca. 40,000 male-specific trichoid sensilla, which respond selectively to pheromone components [Hildebrand and Kaissling, unpublished]. The 2 sensory fibers from each trichoid sensillum terminate in a male-specific region, the macroglomerular complex (MGC), in the antennal lobe (AL) neuropil. All AL neurons that respond postsynaptically to pheromonal stimulation of the ipsilateral antenna have distinctive arbors in the MGC. These cells fall into 2 major classes: local and output neurons. Only the local interneurons have been explored physiologically [Matsumoto and Hildebrand, *Proc. Roy. Soc. Lond. B213*:249, 1981]. We have now physiologically characterized output neurons (ONs) that project to the calyces of the mushroom bodies and the lateral protocerebrum (PC).

Stimulation of the ipsilateral antennal nerve while recording intracellularly from an ON reveals that different neurons belonging to this morphological class may exhibit a variety of complex responses consisting of spikes and subthreshold postsynaptic potentials. For example, one type of ON shows a prolonged inhibition lasting several seconds before resuming regular spontaneous firing at 3-4 Hz. A second type of ON that is normally silent responds with a short-latency (but indirect) excitatory burst (up to 250 Hz). Cells of this type can also be excited, in a dose-dependent manner, by crude pheromone, but not by host-plant odors (e.g. tobacco). An individual ON, however, may be selective for a single pheromone component. We are attempting to correlate this physiological selectivity with particular patterns of dendritic arborization in the MGC.

These results suggest that information about pheromone that is carried out of the ALs and into the PC can take at least 2 forms: spontaneously active outputs are quieted, while silent outputs are excited. Such modulatory mechanisms may be effective ways of fine-tuning the transmission of pheromonal information to higher centers of behavioral control in the CNS. (Supported by an NIH Postdoctoral Fellowship to TC and NIH grant AI-17711 and NSF grants BNS 80-13511 and 83-12769.)

254.21 FINE STRUCTURE OF ANTENNULAR CHEMORECEPTORS IN CAMBARINE CRAYFISHES. Ann Jane Tierney*, C.S. Thompson, L. Marin* and D.W. Dunham* (SPON: H. Atwood). Dept. of Zoology, Univ. of Toronto, Toronto, Ontario, Canada M5S 1A1.

The lateral antennular flagella of decapod crustaceans bear chemoreceptive sensilla called aesthetascs. In the crayfish Orconectes propinquus these sensilla are located ventrally on the 11-13 most distal segments of the lateral flagella. Two clumps of 3-6 aesthetascs occur on each segment, giving a total of approximately 80 aesthetascs/lateral flagellum. Aesthetascs are 100-150 μm long and about 12 μm in diameter. Each has a single annulation 30 μm from the hair base. The sensilla arise from an immovable socket and are directed distally at a 45° angle to the main body of the antennule. Aesthetascs lack an apical pore. However, the distal portion of the sensilla have thin cuticular walls which are readily penetrated by dye and are probably the site where chemical stimuli enter.

In O. propinquus each aesthetasc is innervated by 40-100 sensory neurons whose cell bodies are located approximately 70 μm below the base of each sensilla. Distally each neuron gives rise to a dendrite that develops rootlets and basal bodies and branches into two cilia (diameter .15 - .20 μm). The ciliary structure persists for a short distance and then the microtubules become randomly scattered. Concurrently the dendrite branches dialate to a diameter of .3 - .5 μm. No further branching of outer dendritic segments occurs and thus each aesthetasc contains 80 - 200 sensory endings. At the base of the aesthetasc the dendrites are enveloped by 6 - 7 inner sheath cells which ascend 50 μm into the aesthetasc lumen, sending long finger-like processes around and between the dendrites. The dendrites gradually taper in diameter and terminate 25 μm from the sensilla tip.

The sensory organs of closely related species are expected to be similar in general structure, but the details of morphology and innervation may be influenced by environmental selective pressures such as light conditions or desiccatory or mechanical stress. To determine if crayfish chemoreceptors vary adaptively according to the habitat of the species, we are presently comparing the aesthetascs of O. propinquus to those of crayfishes specialized for life in caves, in terrestrial burrows and in very swift flowing streams.

This study was supported by an operating grant to D.W. Dunham from the Natural Sciences and Engineering Research Council of Canada.

254.22 TEMPORAL ASPECTS OF ZIZIPHINS ACTIONS ON CHEMORECEPTOR CELLS E. S. Peters, Jr.* and L. M. Kennedy, Dept. of Biology, Clark Univ., Worcester, MA 01610.

Ziziphins (Zs) (from Ziziphus jujuba) selectively suppress human sweetness perception (Meiselman et al.,1976), inhibit army worm feeding (Canney & Halpern, 1980), and alter fly behavioral and receptor cell action potential responses to sucrose. The neurophysiological alteration is biphasic: First firing is suppressed; then the firing rate becomes increased and irregular (Kennedy & Halpern, 1979, 1980). After Zs treatment, the onset of the second phase may occur sooner when sucrose is presented continuously than when it is presented at intervals over time (L.M.K.,unpubl. observ., 1979). We studied temporal aspects of Zs actions for continuous vs. intermittent sucrose stimulation in fly (Phormia regina) chemoreceptor cells.

Single chemoreceptor sensilla were given a 1 min Zs (2% aqueous leaf extract) treatment followed by (a) a continuous 5 min sucrose (50mM in NaCl 50mM) stimulation (CS) or (b) an intermittent 5 sec sucrose stimulation every 1 min for 5 min (IS). In control trials, each sensillum was treated with water and then stimulated with sucrose as in the Zs treatment trial for that sensillum. Receptor cell action potentials were tip-recorded extracellularly.

Ratios were formed of the number of action potentials in 100 or 500 msec excerpts taken at 1 min intervals from responses in the Zs treatment trial to the number of action potentials in 100 or 500 msec excerpts taken at the same 1 min intervals from responses in the (control) water treatment trial for each fly. Ratios for CS were initially <1 (median, 0.6,range 0.4-0.7) and then increased to >1 by 3.5 (median,range 1-5) min after stimulation onset. In contrast, ratios for IS, which also were <1 at stimulation onset (median 0.7,range 0.2-0.8), remained <1 (median 0.7,range 0.2-0.9) at 5 min after stimulation onset. Although there was no significant difference between ratios for CS and IS at stimulation onset ($p=0.3$), ratios for CS were significantly greater than those for IS at 5 min after stimulation onset ($p=0.01$). The time required to reach ratios ≥ 1 was significantly less for CS than for IS ($p=0.02$) (Mann-Whitney tests).

Thus the rate of onset of the second phase of Zs actions depends on the amount of stimulation rather than on the absolute time elapsed since Zs treatment. The second phase may be facilitated by receptor cell responding, i.e. be use-dependent. (We thank G. Grubb for leaves. Supported by a Sigma Xi grant to E.S.P. and Clark biology research funds.)

255.1 **CHANNELS FORMED BY DIPHTHERIA, BOTULINUM, AND TETANUS TOXIN IN PLANAR BILAYER MEMBRANES; RELEVANCE TO TRANSLOCATION OF TOXINS INTO CELLS.** D.H. HOCH, A. FINKELSTEIN, L. SIMPSON*, B.R. DasGupta# (SPON: M. Cohen). Dept. Phys. & Biophys., Albert Einstein Coll. of Med., *Dept. of Pharm., Columbia Univ. Med., #Food Research Institute, Univ. of Wisconsin.

Diphtheria, botulinum, and tetanus toxins are thought to consist of two functionally distinct fragments: an active fragment and a binding fragment. In the case of diphtheria toxin, the active fragment has well characterized enzymatic activity; for botulinum and tetanus toxins the precise nature of the active fragment remains unclear. A model has been proposed for the transport of the active fragment of these toxins into the cytosol. The binding fragment attaches to a membrane receptor allowing the whole toxin to be endocytosed into an acidic vesicle. The pH gradient across the vesicle induces a conformational change in part of the binding fragment, forming a transmembrane channel which acts in the transport of the active fragment into the cytosol.

Previous studies have shown that diphtheria toxin inserts into planar lipid bilayers and forms channels that are gated by voltage and pH. Channel formation is maximal when the protein-containing (cis) side is at low pH (4.5) and positive voltages, and the opposite (trans) side is at high pH (7.4); these conditions mimic the pH and voltage gradients across acidic vesicles. An unresolved question has been whether the channel is large enough to allow the active fragment to pass through it. We have shown by selectivity measurements with large anions and cations that at the appropriate pH (selectivity and single channel conductance are a sensitive function of pH) the channel can accommodate ions at least the size of NAD (12-16 Å diameter). This finding is compatible with the channel's proposed function of allowing the fully extended active fragment to pass through it.

We find that tetanus and botulinum toxins show striking parallels to diphtheria toxin in their action on lipid bilayer membranes. Both proteins form channels, and show optimal activity in the presence of a pH gradient (cis 4.5; trans 7). Tetanus toxin channels show voltage-dependent behavior remarkably similar to that of diphtheria toxin.

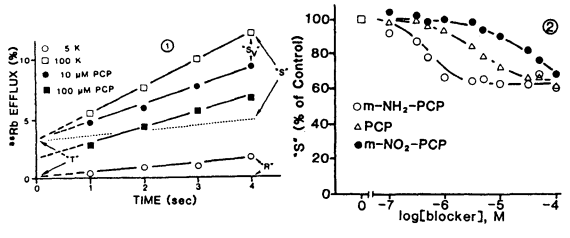
Our findings with lipid bilayer membranes suggest that tetanus and botulinum toxins, like diphtheria toxin, contain an active fragment that is transported across the membrane of acidic vesicles into the cytosol. For all three toxins, the channel formed by the binding fragment may be crucial to the translocation. (Supported by NIH T32GM7288, GM29210-07, NINCDS NS15409, DAMD 17-82-C-2005, DAMD 17-80-C-0100).

255.2 COVALENT LABELLING OF RAT BRAIN MEMBRANE PROTEINS WITH AZIDO-PHENCYCLIDINE (Az-PCP). R.G. Sorensen* and M.P. Blaustein (SPON: B.K. Krueger). Dept. Physiol., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Phencyclidine (PCP) is a widely abused drug that produces a schizophrenia-like syndrome in man. This behavioral effect has been attributed to block of certain K channels in presynaptic nerve terminals (Albuquerque et al., PNAS 78: 7792, 1981; Blaustein & Ickowicz, ibid. 80: 3855, 1983). Physiological (Bartschat & Blaustein, this volume) and biochemical (Sorensen et al., Trans. Am. Soc. Neurochem. 15: 223, 1984) studies suggest that PCP specifically binds to, and blocks certain non-inactivating, voltage-regulated K channels. We therefore utilized the photo-labile analogue of PCP, m-azido-PCP (Az-PCP), which also blocks these channels, to try to identify the proteins that constitute these brain PCP receptors/K channels. Az-PCP was bound to rat brain synaptic membranes (K_D = 200-400 nM) with slightly greater affinity than PCP (K_D = 500-600 nM). Unlabelled Az-PCP competed with ^{3}H-PCP for receptor binding and conversely, PCP displaced bound ^{3}H-Az-PCP, thereby indicating that both compounds interact at the same site(s). When unlabelled Az-PCP was covalently attached (with UV irradiation), and the membranes were extensively washed, subsequent assay for ^{3}H-PCP binding revealed a 50-60% reduction of PCP binding sites. When the membranes were irradiated in the presence of ^{3}H-Az-PCP and washed, subsequent TCA precipitation indicated that the label was covalently attached. These observations show that Az-PCP interacts with the synaptic membrane PCP receptors and can be used to label the receptors. We therefore incubated synaptic membranes with 75-1000 nM ^{3}H-Az-PCP in the dark, then subjected the suspensions to 366 nm irradiation to promote the attachment of the analogue, and prepared the samples for SDS-PAGE and fluorography. A number of polypeptides covalently incorporated the label, including those with apparent M_R of 330K, 100K, 56K, 54K and 50K (which were some of the most heavily labelled). The covalent attachment required photolysis, and the extent was dependent upon irradiation time and Az-PCP concentration. Excess unlabelled PCP (1-10 mM) markedly reduced the incorporation of label into many of the polypeptides, including the five mentioned above. Supported by NIH grant NS-16106.

255.3 PHENCYCLIDINE (PCP), IN LOW DOSES, SELECTIVELY BLOCKS A SPECIFIC PRESYNAPTIC K CHANNEL IN MAMMALIAN CNS. D.K. Bartschat* & M.P. Blaustein. Dept. Physiol., Univ. Maryland Sch. Med., Baltimore MD 21201

Rubidium efflux from ^{86}Rb-loaded rat brain synaptosomes was used to measure the K permeability of the plasma membrane under "resting" conditions (5 mM $[K]_o$) and under depolarizing conditions (elevated $[K]_o$) as described (Neurosci. Abstr. 9: 21, 1983). In the absence of $[Ca]_o$, three pharmacologically distinct components of Rb efflux were observed: i) A K conductance responsible for the resting K permeability ("R"); ii) a transient, depolarization-activated K conductance that was very sensitive to 4-AP and TEA ("T"); and iii) a steady-state depolarization-activated K conductance that did not inactivate in <5 sec. ("S"). Theoretical calculations and pharmacological data indicate that, with $[K]_o$ = 100 mM, 2/3 of "S" represents enhanced efflux through "R" due to the increased driving force on Rb; 1/3 of "S" (= "S_V") represents a separate voltage-regulated K channel. The behavioral and mental aberrations produced by the psychotomimetic, PCP, and related analogues may be a result of blockage of presynaptic K channels (PNAS 78: 7792, 1981). PCP, blocked about 1/3 of the total Rb efflux through "S" (K_I = 1-2 μM), presumably the efflux through "S_V"; "T" was much less sensitive, and "R" was insensitive to PCP (Fig. 1). The relative ability of several PCP analogues to block "S", m-NH_2-PCP > PCP > m-NO_2-PCP (Fig. 2), corresponds to their relative behavioral potency (PNAS, loc.cit.); m-azido-PCP also blocks "S_V". These data suggest that block of a specific class of presynaptic terminal K channels (corresponding to "S_V") may be responsible for the psychotomimetic effects of PCP and related drugs. Supported by NS-16106.



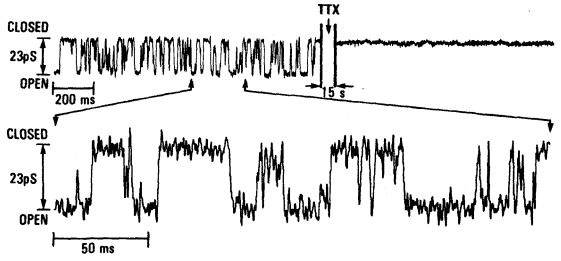
255.4 **RESTORATION OF VOLTAGE-DEPENDENT STATE CHANGE IN THE PURIFIED Na CHANNEL FROM RAT BRAIN.** D.J. Feller, J.A. Talvenheimo*, and W.A. Catterall. Department of Pharmacology, University of Washington, School of Medicine, Seattle, WA 98195.

Sodium channels bind ^{125}I-labeled Leiurus quinquestriatus scorpion toxin ([^{125}I]LqTx) in a voltage-sensitive manner. The K_D increases 10-fold for each 15 to 20 mV depolarization in parallel with Na channel activation (Catterall, W.A., J. Gen. Physiol., 74:375, 1979). Thus, LqTx binding provides a probe of voltage-dependent changes of the functional state of the sodium channel. Na channels purified to homogeneity from rat brain (Hartshorne, R.P. and Catterall, W.A., J. Biol. Chem., 259:1667, 1984) lose this property. Reconstitution of the purified channels into phosphatidylcholine (PC) vesicles restores veratridine-stimulated Na^+ influx (Talvenheimo, J.A. et al, J. Biol. Chem., 257:11868, 1982). However, the addition of brain lipids is necessary to observe [^{125}I]LqTx binding (Tamkun, M.M. et al, J. Biol. Chem., 259:1676, 1984). The objectives of the present study were to determine the minimal brain lipid combination needed to restore [^{125}I]-LqTx binding and to establish conditions under which voltage-sensitivity can be restored. Data are expressed as the ratio of [^{125}I]LqTx bound (mol) to [^{3}H]saxitoxin (STX) bound (mol). [^{3}H]STX binding was assayed at a saturating concentration, while [^{125}I]LqTx binding was determined at a concentration below its K_D. Ratios of LqTx/ STX bound ranged from 0.6 x 10^{-3} to 1.1 x 10^{-3} for PC/brain lipid (40/60, w/w). Reconstitution into PC/phosphatidylethanolamine (PE) (65/35) vesicles restored [^{125}I]LqTx binding at a ratio of 1.2 x 10^{-3} to 1.6 x 10^{-3}. Combinations of other brain lipids with PC were not as effective as PC/PE. Dilution of PC/PE vesicles to form a 40-fold Na gradient (in>out) increased specific LqTx binding 20-fold. Half-maximal [^{125}I]LqTx binding was observed with a 7-fold Na gradient, at a calculated membrane potential of -52 mV (inside negative), in close agreement with data for native Na channels. The increase in binding is due to a decrease in K_D with no change in B_{max}. The K_D was 1.9 nM at -90 mV compared to 4.9 nM at -40 mV. These results show that the purified rat brain Na channel is sufficient for reconstitution of voltage-sensitive [^{125}I]LqTx binding when reconstituted in PC/PE membranes. This suggests that the voltage-dependent gating functions of the purified Na channel are restored upon incorporation into PC/PE vesicles.

255.5 FUNCTIONAL RECONSTITUTION OF THE PURIFIED BRAIN SODIUM CHANNEL IN PLANAR LIPID BILAYERS. R. Hartshorne*+, B. Keller*+, J. Talvenheimo*#, W. Catterall#, and M. Montal+. +University of California San Diego, La Jolla, CA 92093 and #University of Washington, Seattle, WA 98195.

The voltage dependent sodium channel was purified from rat brain and consists of three subunits: α, β1 and β2 (1). The purified protein was reconstituted into liposomes composed of 35% brain phosphatidylethanolamine (PE) and 65% brain phosphatidylcholine (PC) (2). To assay the activity of the Na^+ channel by direct electrical measurements, the reconstituted vesicles were fused with black lipid membranes (3) composed of 1-palmitoyl-2-oleoyl PE and PC (4%:1%) in n-decane. The figure illustrates single Na^+ channel currents activated by 2μM batrachotoxin (BTX) and recorded at a potential of -70mV (electrophysiological convention). A section of the record (filtered at 500Hz) is shown below at faster time resolution. The single channel conductance is 23pS in a 0.5M/0.2M NaCl gradient. Channel openings are abolished by the addition of 3μM tetrodotoxin (TTX). The K_i for TTX inhibition is $\sim 10^{-8}$M at -60mV while at +60mV it is $\sim 10^{-7}$M. Na^+ channel gating is voltage dependent in the presence of BTX with negative potentials increasing the probability of channel closure. The channels are closed 50% of the time at -98mV ± 14mV (n=9). Thus, purified Na^+ channels in planar lipid bilayers display the known single channel conductance, neurotoxin sensitivity and voltage dependence associated with native Na^+ channels.

(1) Hartshorne, R. and Catterall, W. (1984) J. Biol. Chem. 259: 1667-1675; (2) Feller, D. et al. (1984) Soc. Neurosci. Abstr. 10; (3) Krueger, B. et al. (1983) Nature (London) 303: 172-175; Supported by N.M.S.S., E.A.P., N.I.H., and D.A.M.R.



255.6 A FLUORESCENCE ASSAY FOR CATION FLUX INTO LIPOSOMES CONTAINING SODIUM CHANNELS PURIFIED FROM ELECTROPHORUS ELECTRICUS S.A. Tomiko*, R.L. Rosenberg* and W.S. Agnew Dept. of Physiology, Yale University School of Medicine, New Haven, CT 06510

A spectroscopic transport assay based on thallium (Tl^+) quenching of a fluorophore, 8-amino-1,3,6-naphthalenetrisulfonate (ANTS)(Moore & Raftery, PNAS, 77, 1980), has been adapted to measure ion flux through the reconstituted voltage-dependent Na channel. Previously, the Na channel protein purified from eel electroplax was reconstituted into PC liposomes and neurotoxin-modulated ion transport was measured by $^{22}Na^+$ influx (Rosenberg, et al., PNAS, 81, 1984). Tl^+ can pass through activated Na channels (P_{Tl}/P_{Na}=0.33 for normal channels and >1 for batrachotoxin (BTX)-modified channels) to quench fluorescence of internalized ANTS ($\Delta F/F^o$=0-30%). In these studies, the purified protein was supplemented with liposomes of PE/PS/PC (5:4:1 molar ratio) to final concentrations of ~75 ug protein/ml and 10 mg lipid/ml before detergent adsorption. ANTS (30 mM) was internalized by freeze-thaw-sonication. More than 99.9% of the external dye but less than 6% of the vesicles were removed by centrifugation through Sephadex G-50. Fluorescence was measured in a filter fluorometer (excitation 365 nm, emission cutoff 470 nm) with a flowcell of 20 μl illuminated volume, containing 2-20 femtomoles of TTX-binding protein. A Y-connector leading from sample syringes to the flowcell mixed the vesicles with 80 mM $TlNO_3$. Isometric delivery of both solutions required <4 sec; timecourses were recorded after termination of flow. Vesicles with BTX-activated Na channels (5 μM, 45 min at 30°C) exhibited more rapid rates of quench than controls. Signal size was very sensitive to sonication time within 0-5 sec. The BTX-dependent signal was blocked partially by external TTX (1 μM) and completely by dibucaine (0.1 mM). The signal/leak ratio was amplified dramatically and the influx timescale slowed from sec to min when external Na^+ was removed to create a voltage gradient (Garty, et al., JBC, 258, 1983). Then, raising $[TlNO_3]$ from 1-40 mM increased quench at 60 sec in BTX-treated samples up to 3.4 fold above controls; dibucaine blocked the signal. The $K_{1/2}$ for BTX-activation was ~0.3 μM. Veratridine (100 μM) also stimulated quench (Ver. ≃ 35% BTX). This fluorescence assay is ~1000 fold more sensitive than radiotracer assays, and is well suited for large scale screening of drug effects on Na channel activity. Grant support from NMSS and NIH (NS 17928).

255.7 MAINTAINED OPENING OF SINGLE Na CHANNELS BY FENVALERATE. S.F. Holloway*, V.L. Salgado*, C.H. Wu and T. Narahashi (SPON: S-C. CHENG). Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

The effects of the pyrethroid fenvalerate (10 μM) on single Na channels of NIE-115 neuroblastoma cells were studied using the patch clamp method (inside-out configuration) at a temperature of 10-12 °C. The fenvalerate-modified channel, once opened, remained open throughout the entire depolarizing pulse (200 ms) and long after repolarization. The mean open time was about 1 s at -80 mV and exhibited a voltage dependence similar to that of macroscopic tail currents. This type of modified channel activity, designated mode I, can account for the prolongation of Na current during and after a depolarizing pulse under macroscopic voltage clamp conditions. Occasionally, the channel exhibited spontaneous openings at the holding potential. These openings had a mean open time of about 50 ms at -80 mV and occurred in clusters of lasting about 10 s. This type of activity, designated mode II, may explain the increase in holding current seen in macroscopic studies. The two modes of activity do not occur simultaneously.

Macroscopic voltage clamp data have been explained by the open channel modification scheme, in which the pyrethroid is thought to bind to and modify the channel while it is in the open state. At the single channel level, this model predicts that the probability of a channel being modified is zero upon opening and increases with dwell time in the open state. However, if pulses were terminated shortly (< 3 ms) after channels opened, the probability that a channel was modified did not depend on how long it had dwelt in the open state. This result contradicts the open channel modification scheme, and suggests that the channel is modified before it opens.

The modified open channel exhibits properties not normally observed. 1) Transitions to a subconducting state occur, with dwell times as long as 100 ms. 2) The current noise increases while the channel is open, and the difference spectrum (open minus closed) shows a Lorentzian with a cutoff frequency of 200-300 Hz. 3) The current-voltage relation, which could be measured with a voltage ramp pulse during a single opening, shows a decrease in conductance at hyperpolarized potentials, presumably due to block by Ca ions.

Supported by HIH grant NS14143 and Muscular Dystrophy Association Fellowship (S.F.H.).

255.8 RAPID VOLTAGE-DEPENDENT BINDING OF α-SCORPION TOXINS TO NA CHANNELS. G.K. Wang* and G.R. Strichartz. Anesthesia Res. Labs, Harvard Medical School, Boston, MA 02115.

We have examined the voltage-dependent action of a purified Leiurus scorpion toxin (IIα) on Na channels in single myelinated nerve fibers under voltage-clamp. This Leiurus α-toxin specifically slows and prevents the normal fast inactivation from reaching completion, with a concentration dependence showing one-toxin-to-one-Na channel relationship. As a result, a steady-state Na current occurred during a prolonged depolarization lasting at least several seconds. These steady-state Na currents have a different voltage dependence from peak Na currents and appear to result from the reopening of previously inactivated Na channels. The reopening kinetics of the steady-state current are exponential and occur about 100-fold slower than the normal activation process. The effects of Leiurus toxin on slowing of Na channel inactivation processes and on producing steady-state Na currents could be reversed by repetitive depolarizing "conditioning" pulses. This modulation was voltage and duration dependent; higher voltages (≥ + 20 mV) reversed more of slowed Na channel inactivation and longer pulses increased this reversal effect. Using a constant repetitive pulse duration of 100 ms at 2 Hz we found that the maximal reversal effect was saturated around +120 mV, with a voltage-dependence of ~35 mV/e-fold change. A very long pulse (4 sec) was as effective as 40 repetitive pulses of 100 ms applied at 2 Hz. This reversal process does not correlate with the time- or voltage-dependence of channel activation or normal inactivation. Comparable results were also found for sea anemone toxin (ATXII) and Centruroides α-scorpion toxin (IVα) but with differences in the rate of binding as well as the range of voltage-dependence. We propose that the α-toxin binding site, apparently on the external surface of the Na channel, changes its conformation during the fast inactivation process. Leiurus toxin slows and/or prevents the conformational change of the binding site at voltages ≤ 0 mV, due to its high affinity interaction with the binding site. However, at higher voltages the toxin cannot prevent inactivation from occurring during long or repetitive pulses, suggesting that the conformational change caused by the fast inactivation process reduces the toxin binding affinity. Supported by a Multiple Sclerosis Society Grant (RG1513-A-1) to GRS.

255.9 PROPERTIES OF BATRACHOTOXIN-MODIFIED SODIUM CHANNELS IN VARIANT NEUROBLASTOMA CELLS. L-Y.M. Huang. Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, Texas 77550.

The gating properties of sodium (Na) channels of C9 cells were studied in the presence of batrachotoxin (BTX) using whole-cell recording and patch clamp techniques. BTX, a specific activator of Na channels, activated Na channels of NG108-15 cells at hyperpolarized potential (e.g. - 90mV) and eliminated both fast and slow inactivations (Huang et al, PNAS 79:2082, 1982). Na channels of C9 cells could not be activated by depolarization. However, in the presence of BTX, Na channels were activated when the membrane potential was stepped to a membrane potential more depolarized than -80mV. The concentration of BTX used to activate Na channels in C9 cells was usually 1.5-2 fold higher than that used in NG108-15 cells. BTX-activated Na current in C9 cells did not inactivate. This current is very resistant to inhibition of Tetrodotoxin (TTX). TTX at 100μm would block the current through the BTX-activated Na channels in these cells. This TTX concentraction was about 100 fold higher than that required to block the Na channels in NG108-15 cells.

The conductance of single BTX-activated channels is about 12pS. The channels are often open for tens or hundreds of mS. The mean open time of these channels became longer as the membrane potential was depolarized. The histogram of the open state dwell time at membrane potentials -80mV to -40mV could be fitted by single exponentials. Thus the properties of BTX-activated channels in C9 cells were qualitatively similar to those found in NG108-15 cells. (Supported by NIH NS19352-02)

255.10 BATRACHOTOXIN-BENZOATE BINDING AS AN INDEX OF PYRETHROID INTERACTION AT NA+ CHANNELS. G. B. Brown and R. W. Olsen. The Neurosciences Program and the Dept. of Psychiatry, School of Medicine, Univ. of Alabama in Birmingham, University Station, Birmingham, AL 35294, and the Dept. of Pharmacology, School of Medicine, Univ. of California, Los Angeles, CA 90024.

Jacques et al. (BBA 600:882, 1980) recently demonstrated that in cultured mammalian neuroblastoma cells a series of active pyrethroid insecticides synergistically enhance sodium influx stimulated by the channel activators batrachotoxin, veratridine, grayanotoxin and polypeptide scorpion and anemone neurotoxins. These studies prompted us to investigate the effects of a variety of synthetic pyrethroids on the binding of the labeled batrachotoxin derivative, batrachotoxinin-A (^{3}H) benzoate (^{3}H-BTX-B), to mammalian sodium channels. Equilibrium binding of ^{3}H-BTX-B in the presence of excess scorpion toxin to sodium channels in microsac preparations from rat cerebral cortex was measured as described previously (Catterall et al., J. Biol. Chem. 256:8922, 1981; Creveling et al., Molec. Pharmacol. 23:350, 1982). Concentrations ranging from 0.1 to 150 μM of the α-cyano pyrethroids deltamethrin and cypermethrin (8 stereoisomers) and the non-cyano pyrethroids 1R-transpermethrin and tetramethrin were tested for effects on specific ^{3}H-BTX-B binding. Deltamethrin and the two most active isomers of cypermethrin (carboxyl carbon R and cyano carbon S configuration) all enhanced specific binding of ^{3}H-BTX-B 2-3 fold with EC_{50} values of apprx. 1 μM (0.5 - 2 μM). Concentrations greater than 75 μM, however, produced less than the maximal enhancement seen at lower doses. Scatchard analysis of BTX-B binding isotherms in the presence and absence of a saturating concentration of deltamethrin revealed that the enhancement was due to a 3-fold increase in BTX-B binding affinity with no effect on the maximum number of binding sites. In contrast to these active insecticides, the less toxic non-cyano pyrethroids and the remaining six cypermethrin stereoisomers produced a dose-dependent inhibition of ^{3}H-BTX-B binding. Accordingly, permethrin was found to antagonize the enhancement of ^{3}H-BTX-B by deltamethrin.

These studies confirm the suggestion of Jacques et al. (1980) that the toxic pyrethroids bind to sodium channels at a site which is physically distinct from yet coupled to the binding site of channel activators such as batrachotoxin. The correlation between insecticidal activity and enhancement of ^{3}H-BTX-B binding observed for this series of pyrethroids suggests the potential utility of the approach to assess the activity of new synthetic compounds in this important class of insecticides.

The cypermethrin stereoisomers were a gift from Ciba Geigy, Basel. The work was supported by grants NIH-NS-15617 to GBB and USARO-DAAG-29-83-K-0156 to RWO.

255.11 BATRACHOTOXIN ACTIVATES SODIUM CHANNELS OF ADULT FROG SKELETAL MUSCLE. C.N. Allen and E.X. Albuquerque. Dept. Pharmacol. & Exp. Therap., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Batrachotoxin (BTX), a neurotoxin of the Colombian frog Phyllobates aurotaenia, depolarizes nerve and muscle membranes by activating voltage-sensitive Na channels. Single fibers from the interosseal muscle of Rana pipiens were enzymatically dispersed and mounted in a recording chamber using a parafilm-paraffin oil adhesive. Standard patch clamp procedures were used to obtain giga-ohm seals and record single channel currents. Inclusion of BTX (10 nM) in the patch microelectrode resulted in spontaneously occurring unit currents which could be recorded at membrane potentials varying from -10 mV to -110 mV. These currents were not present with only saline in the micropipette and were identified as flowing through Na channels since they were blocked by tetrodotoxin (300 nM). The number of active Na channels in each patch was estimated from the multiple openings to be between 1 and 4, with 2 channels being the most common. The single channel conductance was calculated from the slope of a plot of membrane potential versus channel current to be 19 pS and the reversal potential was estimated to be +20 mV. The open channel was interrupted by many fast closings (flickers) which increased in frequency as the transmembrane potential increased. Histograms of the distribution of channel open times could be fit by a single exponential function indicating a single open state for these channels. The mean channel open time was not dependent on the membrane potential. For example, the mean lifetime as 6.9 ± 1.7 msec (mean ± S.D., n=5) at membrane potentials between -30 and -45 mV. At potentials between -50 and -70 mV, the open time was 7.2 ± 1.76 msec (n=5). The channel lifetime increased slightly to 10.8 ± 2.4 msec at membrane potentials between -90 mV and -110 mV (n=5). These data indicate that BTX depolarizes muscle membranes by activating individual Na channels at membrane potentials where the Na channels are normally closed. Recent evidence suggests that at depolarized membrane potentials Na channels can only open once before closing to an inactivated state. The flickering and repeated opening of the Na channels at a constant membrane potential demonstrate that in the presence of BTX the Na channels do not inactivate. (Supported by USPHS Grant NS-12063 and U.S. Army Med. Res. and Develop. Command Contract DAMD-17-81-C-1279)

255.PO ACTIVITY ASSOCIATED CHANGES IN NODE OF RANVIER ULTRASTRUCTURE. C.C. Wurtz and M.H. Ellisman. Lab. for Neurocytology, Dept. of Neurosci., U.C.S.D., La Jolla, Ca. 92093.

Changes in the compound action potential (CAP), following prolonged activation of frog dorsal roots are accompanied by changes in the microanatomy of the paranodal apparatus. The physiological changes appear as a slowing of the CAP and a reduction in its overall amplitude. Morphometric analysis of fibers orthodromically stimulated at either 2,5,20 or 50hz [for 15 min.] followed by immediate in situ aldehyde fixation, reveals a frequency dependent increase in the size and number of intramyelinic vacuoles. These result from the splitting of the minor dense lines without disruption of the axoglial junctions or alteration of the dimensions of the node proper. This effect is significantly inhibited when the roots are bathed in tetrodotoxin during the stimulation phase and appears to reverse if stimulation is halted and the nerve is allowed to recover prior to fixation. In this case some vacuole reduction is evident after 15 min. and substantial reduction (approaching control values) after 30 min. The time course of structural recovery is paralleled by the recovery of the CAP.

By using computer assisted planimetry of micrographs from longitudinal thin sections the percentage of the paranodal apparatus taken up by vacuoles was determined. A significant 6-fold difference was found between the high frequency (20hz+50hz) group and the controls ($P<.03$), as well as a smaller difference between the high frequency group and the low frequency (2hz+5hz) group. The magnitude of this change also appears to be linearly related to fiber diameter. Large (>12 μm) fibers exhibit the most robust effect, medium (4-8 μm) fibers show a lesser effect and fibers <2 μm are generally uneffected. This indicates that nodes of Ranvier from fibers of different diameters may be differentially susceptible to activity induced morphologic changes or the associated changes in physiology. Our initial analyses of the physiological changes in response to the stimulation also indicate that a more pronounced slowing and alteration of the wave-form arises from the more rapidly conducting fibers. We suspect that large and small nodes of Ranvier are differentially sensitive to spike aftereffects and that this relates to the observed structural changes.

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255.PO CAN PHOTOCHEMICAL REACTIONS ALTER NEURONAL ACTIVITY IN HUMANS? J.B. Walker. Dept. of Psychiat. & Human Behavior, Univ. of Cal. Irvine Med. Ctr., 101 City Dr. So., Orange, Cal. 92668.

All biological effects of optical radiation are due to radiant heating, photochemical reactions, or both. At present, most biomedical applications of laser rely on the fact that exposure to monochromatic light results in in thermal effects (Regan, J.D., and Parrish, J.A. The Science of Photomedicine, Plenum Press), however, photochemical phenomena remain largely unexplored. Such photochemical effects may be relevant in explaining the behavior of different neural preparations after exposure to low power monochromatic light. For example, brief exposure to an argon laser, alters the firing pattern of isolated abdominal ganglion cells of Aplysia (Fork, R.L. Science, 1971; 171: 907-908). These alterations are not due to heating because they occur before there is a detectable increase in temperature. Furthermore, heating does not produce similar biological effects.

We now propose that irradiation of the skin overlying peripheral nerves with a low power helium-neon laser (632.5 nm., 1 mW, 20 Hz.), a procedure which produces no detectable increase in skin temperature, results in depolarization of the underlying nerve in humans. This conclusion is supported by various lines of evidence:

(1) Irradiation of the skin underlying peripheral nerves results in a reproducible somatosensory evoked potential with a latency identical to that observed after electrical stimulaton.
(2) Irradiation alters the latency and amplitude of the H reflex, a monosynaptic spinal cord reflex.
(3) Irradiation alters the threshold for elicitation of clonus in spastic patients, and a dose response curve can be obtained.

We propose a model which postulates the existence of a population of wavelength-specific neuronal chromophores (optically active molecules). Such chromophores, which have been described in other tissues, have several common characteristics: they are all highly resonant ringed structures with a molecular weight of less than 500 (Regan, J.D., and Parrish, J.A. The Science of Photomedicine, Plenum Press). There are a large number of chromophores available in myelin neuronal membranes, and intraneuronally. Interaction of helium-neon laser irradiation with a preset population of chromophores could result in changes in ionic permeability and lead to depolarization via a number of mechanisms. The present results indicate the peripheral nervous system possesses a previously unsuspected degree of photosensitivity. The notion of that generation of an action potential by the wavelength-specific interaction of a set of neurochromophores with light represents an experimentally verifiable model. The therapeutic effects of laser are under investigation (Walker, J.B., Relief from Chronic Pain by Low Power Laser Irradiation, Neurosci. Letts. 1983; 43: 339-344).

ACTIONAL POTENTIALS AND ION CHANNELS IV

256.1 SLOW INACTIVATION OF THE Ca^{2+} CURRENT OF PARAMECIUM IS VOLTAGE-DEPENDENT AND Ca^{2+}-INDEPENDENT T.M. Hennessey* and C. Kung* (SPON: A. Stretton). Lab. of Molecular Biology, Univ. of Wisconsin, Madison, WI 53706

The depolarization-induced Ca^{2+} current of P. caudatum, isolated by using CsCl-filled electrodes and TEA-containing bath, was examined under voltage clamp. This Ca^{2+} current inactivates within tens of milliseconds and the process has been found to be Ca^{2+}-dependent (Brehm and Eckert, Science 202:1203, 1978). In addition to this form of inactivation, we discovered a slow inactivation which develops during depolarizations and is removed upon repolarizations with time constants of tens of seconds.

The development of this slow inactivation depends on voltage. The time constant of the development is large (about 100 sec) for a depolarization of +20 mV and smaller (about 40 sec) for a +40 mV step. It is independent of large increases in intracellular Ca^{2+} concentration. Injection of EGTA, sufficient to block most of the Ca^{2+}-dependent Ca-channel inactivation, does not affect the kinetics of this slow inactivation.

The removal of Ca-current inactivation after prolonged depolarization shows two distinct kinetics. A portion of the current returns exponentially with a time constant of hundreds of msec (the removal of the Ca^{2+}-dependent inactivation) while the remainder returns with time constants of tens of seconds. The removal of the slow inactivation is also Ca^{2+}-independent since it occurs in EGTA-injected cells. When W-7 is applied along with EGTA during a depolarization and is removed during the test pulses later, there is also no effect on the kinetics of the recovery from the slow inactivation. (W-7 is a drug recently found to block the Ca-current of Paramecium. Hennessey and Kung, J. exp. Biol. 1984, in press).

The physiological and behavioral roles of this slow inactivation will be discussed.

Supported by NSF BNS8216149, PHS GM22714 and NIH NS 06950.

256.2 STIMULATION OF cAMP-DEPENDENT PROTEIN PHOSPHORYLATION RETARDS BOTH INACTIVATON AND 'WASHOUT' OF Ca CURRENT IN DIALYZED HELIX NEURONS. J.E. Chad* and R. Eckert. Department of Biology, UCLA, Los Angeles, CA 90024

The voltage-activated Ca current, I_{Ca}, was examined under voltage clamp in dialyzed Helix aspersa neurons to investigate a possible relationship between Ca-dependent inactivation of I_{Ca} and the 'washout' of I_{Ca} during dialysis. The control dialysate contained 135 mM Cs aspartate, 10 mM Hepes, pH 7.3, and 10 mM TEA, a Ca/EGTA buffer set to 0.1 μM free Ca_i. Cells were bathed in 10 mM $CaCl_2$, 5 mM $MgCl_2$, 35 mM Tris Cl, pH 7.3, 50 mM TEA plus 5 mM 4 AP or 2.5 mM 3,4 DAP at 17-18°C. I_{Ca} was elicited every 60 s with a 70ms voltage step to +10 mV from a potential of -40 mV; leak corrections were made by digital addition of current recorded during equivalent hyperpolarizing pulses. Replacement of external Ca with Mg completely eliminated the inward current, revealing no time-dependent current. During dialysis with control solution, peak I_{Ca} showed the usual decline (washout), dropping to ≈50% in 15 min. External 100 μM forskolin, an activator of adenylate cyclase, produced a temporary reversal of washout in cells dialyzed with Mg-ATP-supplemented control solution, similar to the reversal produced by addition of dibutyryl cAMP. This suggests the presence of an active cyclase in the dialyzed neuron, which may account for separate observations that Mg-ATP slows washout without addition of cAMP. I_{Ca} was sustained or the wash-out partially reversed by introduction of the catalytic subunit (CS) of cAMP-dependent protein kinase (≈15μg/ml protein) in the presence of 4 mM Mg-ATP, confirming independent observations of Doroshenko et al. (Neuroscience 11:263, 1984). With I_{Ca} blocked with 1 mM Cd, addition of CS produced no measurable assymetric effect on the time-independent residual currents. The rate of inactivation during a depolarization was measured in records of similar peak I_{Ca} recorded with fixed depolarizing steps during different phases of such experiments. In each case, addition of CS or other agents promoting kinase activity was accompanied by a slowing of the rate of inactivation. Thus, washout and inactivation of I_{Ca} appear to be related, both being retarded by addition of factors that promote cAMP-dependent phosphorylation. These findings suggest that i) 'washout' results from a progressive dephosphorylation of channel-associated protein, ii) phosphorylation is required to permit Ca channel activation, and iii) Ca current inactivation may involve a dephosphorylation. USPHS NS 8364.

256.3 ELECTROPHYSIOLOGICAL EFFECTS OF PHORBOL ESTER AND PROTEIN KINASE C ON THE BAG CELL NEURONS OF APLYSIA. S.A. DeRiemer, K.A. Albert*, J.A. Strong*, P. Greengard, and L.K. Kaczmarek. Dept. of Pharmacology, Yale Univ., New Haven, CT 06510 & The Rockefeller Univ. N.Y., N.Y.10021.

The bag cell neurons of Aplysia undergo a complex series of changes in their electrical properties during and subsequent to a 30 min. afterdischarge that can be elicited by brief electrical stimulation. Evidence indicates that cAMP-mediated protein phosphorylation plays a role in regulating potassium channels during these changes. Several of the changes may also be related to an elevation of intracellular calcium ions. We have previously shown that there are at least three calcium-dependent protein kinases present in these cells, including the calcium/phosphatidylserine dependent protein kinase (Protein kinase C) which can be stimulated by the tumor promoter 12-phorbol-13-myristate acetate (PMA,TPA). We have now tested the effects of PMA on the electrical activity of bag cell neurons.

Isolated bag cell neurons, maintained in primary cell culture, were exposed to PMA (1-100 nM) while monitoring the electrical properties of the cells with standard intracellular recording techniques. PMA at these doses consistently produced an increase in the height, but not the width, of action potentials evoked by depolarizing current pulses (n=13; mean increase = 28%). In contrast, elevated concentrations of cAMP increase both the height and the width of action potentials in these cells.

In order to determine whether the effects of PMA were due to the activation of protein kinase C, purified enzyme prepared from bovine brain was pressure injected into cultured bag cell neurons. Electrodes were filled at the tip with protein kinase C (18 µg/ml) in a carrier solution of 20 mM potassium phosphate buffer, pH 7.2 and 0.9 M KCl. Following injection, an increase in action potential height was seen in 43% of the cells injected (n=14). The mean increase in height for the cells that responded to injection of the kinase was 26%. No increase in the height of action potentials was observed with injection of heat-inactivated enzyme (n=3) or carrier solution alone (n=3). These results suggest that injection of protein kinase C can produce effects that are qualitatively similar to those of exposing the cells to PMA, and that protein phosphorylation by protein kinase C, as well as by the cAMP-PK, may play a part in controlling the excitability of the bag cell neurons.

256.4 STRONTIUM IONS INDUCE PAROXYSMAL SPIKE BURSTS IN NON-PACEMAKER MOLLUSCAN NEURONS. K. J. Futamachi*, (spon: K. Courtney) Lab Neurophysiology, NINCDS, Nat'l. Inst. Health, Bethesda, Maryland 20205

Strontium (Sr^{++}), a divalent cation which closely resembles calcium in molecular and hydrated ion size, was studied as a substitute for Ca^{++} in neurons of the land snail Helix aspersa. Experiments were performed to study the role of K^+ currents in spike burst generation since CA^{++} is known to activate K^+ currents, whereas Sr^{++} has been shown to block K^+ channels in some preparations. A select group of non-pacemaker molluscan neurons were used for this study because Sr^{++} induces spike burst generation and has a marked effect on the membrane characteristics of this subpopulation of non-pacemakers.

In normal snail Ringers solution, these cells are characterized by action potentials of 10-30 mV and durations of 1-2 msec. The resting membrane potentials (RMP) ranged from 30-60 mV and input resistance (R_N) from 10-30 Mohms. When snail Ringers containing Sr^{++} in place of Ca^{++} replaced the bathing medium, action potential amplitudes and R_N were increased in a manner positively correlated to the extracellular Sr^{++} concentration. RMP was not affected significantly. The normal spontaneous single spike firing pattern was replaced by an increased frequency of single spike firing and by randomly occurring bursts of spikes. The spike burst durations lasted from 0.5 to 15 sec and spike frequencies during the bursts reached rates of 100 Hz.

Two electrode voltage clamp techniques showed that depolarizing and hyperpolarizing command voltage steps produce non-linear but always positive current-voltage (I-V) curves in normal snail Ringers. In contrast I-V curves obtained in Ringers containing Sr^{++} with the same depolarizing command voltage steps show a region of negative slope resistance between -40 and -15 mV. This negative slope resistance reached values of about 10 nA. The magnitude of these negative slope currents was correlated with extracellular strontium concentrations.

These data suggest that Sr^{++} crosses the cell membrane through the Ca^{++} ion channels carrying at least part of the inward current. Sr^{++} also appears to block K^+ channels in this preparation. These two parameters, inward Sr^{++} current and increased R_N due to K^+ channel block, can contribute much toward the enhanced excitability observed when Sr^{++} replaced Ca^{++} in the bathing medium of this molluscan preparation.

256.5 PROPERTIES OF THE PERSISTENT CALCIUM CURRENT OF APLYSIA PACEMAKER NEURONS. Richard H. Kramer* (SPON: R. Zucker) Dept. Physiology, Univ. of Calif., Berkeley CA 94720.

Molluscan bursting pacemaker neurons have a persistent inward Ca^{2+} current which underlies the negative resistance region in the pacemaker potential range of their steady-state I-V relations (see Eckert & Lux (1976) J. Physiol. 254:129-151). I have examined the properties of the persistent inward current in axotomized, voltage-clamped, left upper quadrant bursting (LUQB) neurons from Aplysia abdominal ganglion.

The persistent inward current of LUQB cells is reduced by about 20% following removal of Na^+ from the bathing medium. The effect of Na^+ removal is irreversible, however, and not seen in cells pre-injected with EGTA. The current is blocked by substitution of Co^{2+} or Mn^{2+} for external Ca^{2+}. Thus, the persistent inward current is a Ca^{2+} current.

The voltage-dependence of the persistent inward current was studied by using constant (5 mV) hyperpolarizing pulses from hold potentials between -35 and -75 mV to deactivate a portion of the resting persistent Ca^{2+} conductance. Hyperpolarizing pulses originating from near -35 mV result in a net outward current due to the deactivation of a large steady-state Ca^{2+} conductance. The deactivation has complex kinetics, including a slow component which develops over several seconds. Pulses originating from more hyperpolarized levels result in much less deactivation. As judged from its deactivation by hyperpolarizing pulses, the persistent Ca^{2+} current activates steeply between -50 and -35 mV.

The voltage-dependence of the persistent Ca^{2+} current was also studied by generating steady-state I-V curves using prolonged (2 sec) pulses to potentials between -30 and -80 mV. 50 mM TEA was added to the bathing medium in order to block the Ca^{2+}-dependent K^+ conductance. Subsequent substitution of Co^{2+} or Mn^{2+} for Ca^{2+} resulted in a voltage-dependent outward shift in the I-V curve above -50 mV. Thus, LUQB cells have a large steady-state Ca^{2+} current (5-10 nA) flowing at a potential equal to their average resting potential (about -40 mV).

The steady-state Ca^{2+} current is inactivated by Ca^{2+}. Intracellular Ca^{2+} injection elicits an early, TEA-insensitive outward current which is blocked by external Co^{2+}. Ca^{2+} injection, and depolarizing prepulses which elicit Ca^{2+} influx result in a decrease in the persistent inward current activated by subsequent depolarizing pulses. The recovery from Ca^{2+}-dependent inactivation takes tens of seconds. The Ca^{2+}-dependent inactivation of the persistent Ca^{2+} current underlies the inter-burst hyperpolarization of the unclamped bursting cell. Supported by NIH grant NS 15114.

256.6 DECREASED CONDUCTANCE INWARD CURRENTS PRODUCED BY THE 8-BROMO DERIVATIVES OF cAMP and cGMP IN APLYSIA INK MOTOR NEURONS AND TAIL SENSORY NEURONS. John P. Walsh and John H. Byrne, Dept. of Physiology and Cell Biology, Univ. Texas Med. Sch. at Houston, Houston, TX 77030

For both the tail sensory neurons in the pleural ganglia and L14 ink motor neurons in the abdominal ganglion serotonin (5-HT) produces a slow decrease in a resting potassium conductance that appears to be mediated by changes in levels of cAMP. Previously, we reported that the adenylate cyclase activator forskolin could both mimic the response to 5-HT and block further responses to 5-HT in these neurons (Walsh and Byrne, 1983).

To examine further the role of cyclic nucleotides as second messengers in these cells we bath applied the 8-bromo derivatives of cAMP and cGMP to each preparation. Addition of 8-bromo cAMP (10^{-3}M) and 8-bromo-cGMP (10^{-3}M) to the L14 cells each produced a large inward current associated with a decrease in the input conductance. Responses produced by 1-2 second pressure ejection of 5-HT from a micropipette were blocked only in those cells exposed to 8-bromo-cAMP and not in cells exposed to 8-bromo-cGMP. Although 8-bromo cGMP had no effect on the 5-HT response, the similarity in current and conductance change to that produced by both 5-HT and 8-bromo-cAMP suggests that different internal mechanisms can generate parallel forms of cellular response. Such a redundancy of response expression would allow for an additive integration of afferent information, provided each internal messenger was increased through seperate extracellular signals.

Addition of the same concentrations of the 8-bromo derivatives of cAMP and cGMP to the tail sensory neurons also produced an inward current and decrease in input conductance. The independent effects of these agents upon the tail sensory neurons' response to subsequent application of 5-HT using micropressure ejection techniques is currently under investigation, as well as the effective concentrations of these compounds needed to both mimic and modulate the 5-HT responses.

256.7 NIFEDIPINE BLOCKADE OF NEURONAL CALCIUM CURRENTS. A. M. Gurney and J. M. Nerbonne. Biology Division, Caltech, Pasadena, CA 91125.

In order to evaluate the cellular specificity of nifedipine blockade of Ca^{++} currents and to study its mechanism of action, we have examined the effects of this calcium antagonist on neuronal calcium currents. Enzymatically dissociated bag cells, isolated from the marine mollusc Aplysia californica, were maintained in supplemented artificial sea water (ASW) at 12-14°C. For recording, cells were placed in ASW (15°C) containing 5 µM TTX to block the fast Na^+ current. Pipettes contained 480 mM CsCl to suppress K^+ currents. In whole-cell recordings from cultured bag cells, nifedipine reduced the amplitude of the slow inward Ca^{++} current (I_{Ca}) with IC_{50} ~1 µM. As in other preparations, nifedipine blockade did not display voltage- or "use"-dependence. Although the amplitude of I_{Ca} was reduced in 0.2-10 µM nifedipine, the rate of activation of the current was unaltered at all concentrations and at all voltages tested (-10 to +50 mV), consistent with nifedipine binding to and blocking the resting, closed state of the channel. At all voltages, the rate of inactivation of I_{Ca} was accelerated in a dose-dependent fashion, consistent with the additional presence of open-channel blocking activity. These effects are qualitatively and quantitatively similar to previous observations on nifedipine blockade of I_{si} in mammalian heart muscle. In contrast, we were unable to observe any effects of nifedipine, at concentrations up to 10 µM, on whole-cell Ca^{++} currents recorded from cultured ciliary (chick) or superior cervical ganglion (rat) neurones.

The nifedipine molecule, which contains an o-nitrobenzyl moiety, is photolabile and irradiation destroys its blocking activity. Nifedipine induced suppression of I_{si} could be removed only very slowly by washing with drug-free solutions, but the process could be accelerated with light-flashes of 1 ms duration delivered from a xenon flash lamp. A single flash, however, resulted in recovery of less than 10% of the current amplitude; multiple flashes were required for complete recovery of the current. It seems that this effect probably arises from the high degree of pigmentation present in bag cells and suggests that the drug might act at sites other than the extracellular surface of the plasma membrane.

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256.8 TETRODOTOXIN RESISTANT PROPAGATING CALCIUM SPIKES IN IDENTIFIED NEURONS OF THE GIANT BARNACLE, Balanus nubilus L.A.Lewenstein and W.N. Ross, Dept. of Physiology, New York Medical College, Valhalla, New York 10595.

Calcium channels have been demonstrated in axons, but they are of such a low density that outward potassium current must be blocked in order to create an all-or-none calcium spike. The cross-commissural cells in the supraesophageal ganglion (one soma located on the posterio-medial margin of each hemiganglion with an axon projecting out the contralateral antennular nerve) are unique in that the concentration of calcium channels in their axons is sufficient to support TTX-insensitive propagation in either direction in sodium-free saline without the addition of 4-AP or TEA.

In normal saline, the cell produces action potentials with an average amplitude of 80 mV, a 4 msec duration at half-height and a 60 msec undershoot. Although the spike is mixed under normal physiological conditions, the sodium component can be eliminated with 0.3 µm TTX or by substitution with choline chloride. The remaining all-or-none calcium component recorded in the soma is an average of 30% smaller in height and 41% wider than the mixed spike. There is considerable variation among individuals, but the calcium component will propagate the entire length of the neuron in all of them.

The calcium component is blocked by 10mM Co, 1mM La, 1mM Cd and 1mM Ni. 10mM Mg does not block propagation. 20 mM Sr or Ba may be substituted for calcium to elicit a regenerative response. Barium has the added effect of maintaining long depolarizations which last for several seconds before returning to resting levels.

The location of calcium channels in this neuron was examined by looking for the site of stimulus-induced absorbance changes after injecting the cell with Arsenazo III. These measurements have confirmed calcium entry into the soma, along the length of the axon across the commissure, through the contralateral hemiganglion and out the antennular nerve. The amount of calcium influx is not significantly altered when TTX is added to normal saline.

Normal barnacle saline contains 20 mM calcium. Intracellular recordings from graded calcium experiments have revealed that the critical amount of calcium needed to support propagation in TTX is less than 5mM. Propagation has been observed after exchanges as low as 1.25 mM, but at these low concentrations the cell rapidly depolarized (less than 5 minutes) and was irreversibly damaged.

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256.9 BENZODIAZEPINE INHIBITON OF CALCIUM CONDUCTANCE IN IDENTIFIED LEECH NEURONS. J. Johansen, W.C. Taft*, J. Yang, R.J. DeLorenzo, and A.L. Kleinhaus, Dept. Neurology, Yale U Sch. of Med., New Haven, CT 06510.

Benzodiazepines (BZs) inhibit voltage sensitive Ca uptake in mammalian nerve terminal preparations and evidence indicates that this action of BZs is mediated by micromolar affinity and not by nanomolar affinity BZ receptors (Taft & DeLorenzo, PNAS:81, 1984). To determine the effects of BZs, on Ca conductances in intact neuronal preparations, we studied the actions of these compounds on leech neurons.

Standard electrophysiological techniques were used to examine the effects of BZs on voltage-dependent divalent cation potentials recorded from nociceptive neurons in Na-free TEA solutions. The amplitude and duration of these long lasting (sec) regenerative potentials depended on $(Ca)_o$ and $(Sr)_o$; they were reversibly blocked by Mn and Co and were resistant to 50 uM TTX a concentration which abolished the Na-dependent action potential(AP) in the same neurons. Medaze - pam, Clonazepam, RO5-4864 and Diazepam all inhibited these potentials in a dose-dependent manner in uM concentrations. The dose-response curve obtained for Medazepam demonstrated that the inhibition began at a concentration of 1-10 uM with a K_I of about 35 uM. Qualitatively similar inhibition of the prolonged divalent cation potentials was obtained with Mn (K_I, 100 uM). 200 uM Medazepam had no effect on the resting membrane potential nor on the maximum rate of depolarization of the Na-dependent AP in normal Ringer. The undershoot of the Na-dependent AP was, however, reduced during drug exposure presumably due to a decreased contribution of gK(Ca). These results suggest that BZs inhibit a specific Ca-conductance in a similar manner to that of Mn, and that the current underlying the prolonged potentials is not passing through the Na channel.

We have obtained evidence that micromolar BZ binding sites are present in leech membranes. The binding curve for 3H labeled Clonazepam shows saturable specific micromolar binding with a K_D of 40 uM and the curve closely parallels that obtained for CNZ photoaffinity labeling to micromolar BZ receptors in synaptosomes. In addition, the K_D obtained from the binding curve in leech membranes is in close agreement with the K_I determined from the electrophysiological experiments.

These findings are consistent with the hypothesis that in the leech BZs specifically bind to micromolar affinity receptors which modulate Ca channels as they do in mammalian synaptosomes.

256.10 ANALYSIS OF CALCIUM-SENSITIVE MEMBRANE CURRENTS IN SYMPATHETIC NEURONS OF BULLFROG. Stephen M. McCort* and Forrest F. Weight. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD. 20852.

Early and late calcium-sensitive currents activated by depolarizing steps to membrane potentials positive to -35 mV have been reported in bullfrog sympathetic neurons (Nature 300: 185, 1982; J. Physiol. 332: 223, 1982). We have studied calcium-sensitive membrane currents over a range of membrane voltages using the single-electrode voltage-clamp technique (Neurobiol. 6: 411, 1975). Type B neurons in the IXth or Xth paravetebral sympathetic ganglion of bullfrog were clamped at a holding potential of -50 mV using 3 M KCl electrodes having resistances between 20 and 30 megaohms. Two types of voltage step commands were used to activate membrane currents: (1) steps one sec in duration from a holding potential of -50 mV to membrane potentials of -20 mV, -35 mV, -65 mV, -80 mV, -95 mV and -110 mV; and (2) a sequential series of voltage steps one sec in duration as follows, -25 mV to -35 mV, -35 mV to -50 mV, -50 mV to -65 mV, -65 mV to -80 mV, -80 mV to -95 mV, and -95 mV to -110 mV. Current-voltage curves constructed from data obtained by both methods were similar. Calcium sensitivity was tested using a Ca^{2+}-free Ringer's solution with 4 or 10 mM Mn^{2+} replacing Ca^{2+}. A slow outward current activated between -50 and -20 mV was significantly reduced in the Ca^{2+}-free/Mn^{2+} Ringer. In some cells, a small slow inward current was elicited by hyperpolarizing steps to -65 mV and -80 mV. That current was greatly reduced or abolished in the Ca^{2+}-free/Mn^{2+} Ringer. The results suggest that Ca^{2+}-sensitive membrane currents can be activated over a range of membrane potentials from -20 to -80 mV.

256.11 CARBACHOL AND BAY K 8644 STIMULATED CALCLIUM UPTAKE INTO CULTURED CHICK MYOTUBES AND BC3H1 CELLS. H. Smilowitz and J.G. Sarmiento*, Depts. of Pharmacology and Medicine, U. of Conn. Health Center, Farmington, CT 06032.

Methods have been developed to measure carbachol stimulated calcium uptake into cultured chick embryo pectoral muscle cells grown on plastic dishes and coverslips. Maximal uptake (40-80 nmoles calcium/mg cell protein containing ~ 1.0 pmol AchR) was into myotubes but not fibroblasts and was achieved within 10-20 seconds with 50 μM carbachol at 37°C. Ca^{++} uptake was via the acetylcholine receptor since it was (a) inhibited by α-bungarotoxin, (b) inhibited by prior exposure to carbachol, (c) competitively inhibited by NaCl (K_i = 1.6 mM) and KCl, and (d) proportional to the number of α-bungarotoxin binding sites on the cells. Surprisingly, the calcium antagonists D600, verapamil and diltiazem potently inhibited carbachol stimulated calcium uptake (IC_{50} = 240, 720, 130 nM, respectively) but nifedipine was only inhibitory in the 10 μM range.

The Ca^{++} channel agonist BAY K 8644 also stimulated ^{45}Ca uptake into the myotubes but not fibroblasts. Maximal stimulation was achieved in two minutes in the presence of α-bungarotoxin (5-10 nmoles calcium/mg cell protein) using 100 nM agonist ($K_{0.5}$ ≃ 50-65 nM) and 0.5 mM Ca^{++}. Agonist stimulated Ca^{++} uptake occurred in the presence of 140 mM NaCl and 5 mM KCl; however, KCl (5-50 mM) has thus far failed to either directly stimulate Ca^{++} uptake or to potentiate 8644 stimulated Ca^{++} uptake into the chick myotubes. Nifedipine and nisoldapine inhibited BAY K 8644 stimulated Ca^{++} uptake in the nM range ($K_{0.5}$ nifedipine was 0.8 - 4.5 nM and for nisoldapine, 5.3 nM). Preliminary binding studies with ^3H nisoldapine indicated a single class of binding sites (B_{max} = 1.1 pmoles/mg protein, K_D = 2 nM) suggesting that the sites to which the 1,4-dihydropyridine agonists and antagonists act in these cells may be identical.

Preliminary developmental studies have shown that carbachol and 8644 stimulated Ca^{++} uptake was detectable on the second and third days, respectively, after plating. Further, while the smooth muscle derived clonal cell line BC3H1 exhibited high levels of carbachol stimulated calcium uptake and α-bungarotoxin binding sites as expected, no 8644 stimulated Ca^{++} uptake and very low levels of basal Ca^{++} uptake was observed. Supported by USPHS NS13860.

256.12 THE MECHANISM OF BINDING OF DIHYDROPYRIDINE CALCIUM CHANNEL BLOCKERS TO BRAIN MEMBRANES. G. A. Weiland and R. E. Oswald. Department of Pharmacology, Cornell University, Ithaca, NY, 14853.

Radiolabeled calcium channel blockers of the dihydropyridine family have been used to label putative voltage-dependent calcium channels from a number of tissues. These studies have yielded important information about the binding of calcium antagonists, including the absolute requirement for divalent cations for binding and the allosteric interactions between nitrendipine, verapamil, and diltiazem. In a first step toward understanding the linkage between binding and its functional consequences, kinetic and equilibrium studies of the binding of two radiolabeled dihydropyridine calcium antagonists to rat brain membranes were performed. [^{3}H](±)Nitrendipine, a racemic mixture, and [^{3}H](+)PN200-110, an optically pure isomer, were utilized in these studies, and their binding properties compared. Analyses of equilibrium binding revealed only a single component with the same number of binding sites for both radioligands. The dissociation rates were measured after various times of association. After short association times [^{3}H](±)nitrendipine dissociation was clearly biphasic, becoming less so with increasing times of association. The dissociation of the optically pure radioligand, [^{3}H](+)PN200-110, however, was monophasic at all association times. The biphasic dissociation observed with [^{3}H](±)nitrendipine is consistent with the two optical isomers of nitrendipine binding with the same association rate but dissociating with a 17-fold difference in rate. The association rates were studied over a 40-fold range of concentration of each radioligand. For both radioligands the associations were biphasic with the rates of the faster components dependent on radioligand concentration and the rates of the slower components independent of radioligand and concentration. These results are consistent with the existence of two interconvertible forms of the putative calcium channel in the membrane, one which binds the radioligands with high affinity in a simple bimolecular reaction (60% of the sites prior to ligand addition) and one which has essentially no affinity for the ligands. The calcium antagonists stabilize the high affinity conformation of the channel, converting all the channels to this form at high concentrations. (Supported by the PMA Foundation and NIH-BRSG 08-57 RR05 462 E-21.)

256.13 POTASSIUM-STIMULATED CALCIUM UPTAKE BY RAT BRAIN SYNAPTOSOMES: INHIBITION BY SULFHYDRYL REAGENTS. Karey E. Eason* and Robert S. Aronstam (SPON: B.D. Goldstein). Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912.

Depolarization of nerve terminals induces an influx of calcium ions which participates in the release of chemical neurotransmitters. Depolarization-induced calcium uptake was measured in synaptosomes isolated from rat forebrain using ^{45}Ca as a radiotracer and elevated K^+ concentrations to decrease resting membrane potential. Ca uptake was more than doubled when synaptosomes were diluted with an influx medium containing 70 mM K^+ and 0.1 mM $CaCl_2$. This uptake was rapid (< 30 sec), saturable, and susceptible to inhibition by divalent cations, including Co^{2+}, Mn^{2+} and Cd^{2+}, which have been shown to block Ca currents in electrophysiological studies.

K^+-stimulated Ca uptake was inhibited by pretreating the synaptosomes with N-ethylmaleimide (NEM), a sulfhydryl alkylating reagent. Incubation of synaptosomes with NEM at 37° for 30 min decreased K^+-stimulated Ca uptake by 7% at 0.1 mM and 90% at 10 mM. Exposure to methylmercury chloride and mercuric chloride similarly inhibited calcium uptake. Inhibition by mercuric chloride was complete at 1 mM. Resting Ca uptake was depressed 25-40% by all of these reagents at 1 mM. Ethylmercury chloride was considerably less potent than the other mercurials at inhibiting calcium uptake.

The content of exposed sulfhydryl groups in the tissue (determined under non-denaturing conditions) was 24 ± 1 nmoles/mg protein. The total sulfhydryl content of the tissue, measured after solubilization in 2% sodium dodecylsulfate, was 55 ± 1 nmoles/mg protein. The content of surface sulfhydryl groups was decreased upon treatment with methylmercury or NEM at the same concentrations at which they inhibited K^+-stimulated Ca uptake. Ethylmercury decreased surface sulfhydryl content by only 10-15% at 1 mM. The influence of mercurials and NEM on K^+-stimulated Ca uptake cannot be unambiguously ascribed to a mechanism containing an essential sulfhydryl-moiety. It is possible that the concentration of the reagents was buffered by the sulfhydryl content of the membranes, depressing their effective concentrations at the relevant site of action.

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256.14 Identification and Pharmacological Characterization of Calcium Channels in Rat Brain Synaptosomes. S.M.Goldin and T.J.Turner*. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

The voltage-sensitive Ca^{+2} channel is an important pathway for Ca^{+2} entry into neurons, smooth muscle, and cardiac tissue. Nifedipine is a potent blocker of Ca^{+2} channels in smooth and cardiac muscle, and binds with high affinity to homogenates of these tissues. While nifedipine binding to brain homogenates is similar to binding in smooth and cardiac muscle, others have reported that it did not block synaptosomal Ca^{+2} uptake or catecholamine release. The lack of nifedipine sensitivity cast doubt upon the existence of functional Ca^{+2} channels in synaptosomes.

We show that rat brain synaptosomes contain functional Ca^{+2} channels that are inhibited by organic Ca^{+2} channel blockers. Depolarization of synaptosomes with high K^+ stimulates uptake of $^{45}Ca^{+2}$ which is biphasic in its time course. Replacement of external Na^+ with choline eliminates the slower phase, leaving only a rapid uptake process that terminates within 1 sec. This rapid, tetrodotoxin-insensitive Ca^{+2} uptake can be inactivated by prior depolarization. Depolarization has no effect on the rate of $^{22}Na^+$ efflux. These results rule out Na^+/Ca^{+2} exchange as a mediator of the rapid phase of Ca^{+2} uptake.

30-50% of the rapid phase of Ca^{+2} uptake is inhibited by nitrendipine, as is 60-80% of depolarization-stimulated norepinephrine release. In physiological external Na^+, the K_i for nitrendipine inhibition of Ca^{+2} uptake is 56 nM. The potency of nitrendipine is increased in the absence of external Na^+ (K_i=1.7 nM), such that inhibition correlates more closely with the binding of ^{3}H-nitrendipine to synaptosomes (K_d=0.35nM). Other organic channel blockers inhibit a similar proportion of the rapid Ca^{+2} uptake (K_i, in nM: nifedipine, 63; verapamil, 310; D600, 150; diltiazem, 210). The potency of these drugs is also increased 10-30 fold in the absence of external Na^+ (K_i, in nM: nifedipine, 4.4; verapamil, 11.2; D600, 12.9; diltiazem, 12.7). The potencies of all Ca^{+2} channel blockers tested by us are in reasonable agreement with their potencies observed in smooth and cardiac muscle. The results are consistent with nitrendipine blockade of Ca^{+2} channels as a result of occupancy of the ^{3}H-nitrendipine binding site, and suggest a single channel flux of 1.4×10^6 ions/sec.

256.15 ION CHANNELS IN HUMAN RETINAL PIGMENT EPITHELIUM.
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The retinal pigment epithelium (RPE) lies just distal to the photoreceptor outer segments and regulates the flow of ions, nutrients, fluid, and vitamin A between the blood supply and the retina. Previous investigations (Miller and Steinberg, J. Memb. Biol. 36:337, 1977) suggested that both the apical and basal membranes of RPE cells are selectively permeable to K^+, but such studies have been limited by the difficulty of recording from an intact epithelial syncitium. To investigate in more detail the ionic conductances of RPE cells, we have used patch clamp methods to record from human RPE cells in culture. Cells obtained from eyes removed during autopsy were dispersed and grown on glass coverslips coated with swine-skin collagen in 1:1 medium 199 and DMEM supplemented with 1% calf serum and 1% bovine retina extract. All recordings were made from inside-out, cell-free membrane patches. Although some patches revealed no channel activity, most showed transitions of at least three kinds: two selective for K^+ with mean conductances near 30 and 100 pS, and a non-selective conductance near 300 pS. None of these showed any obvious voltage dependence of channel gating. The transition rates of the larger K^+ channels and the non-selective channels also appeared unaffected by Ca^{++} concentrations as low as 10^{-8} M at the internal (cytosolic) membrane surface. A current record showing transitions from at least two kinetically different closed states for the large K^+ channel is shown below. Several channels were present in this patch.

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256.16 SINGLE CHANNEL CURRENTS IN PATCHES OF PLASMA MEMBRANE FROM HUMAN MACROPHAGES.
F.V. McCann, S.R. Stibitz*, H.F. Woehlck* and P.M. Guyre*. Dept. of Physiology, Dartmouth Medical School, Hanover, NH 03756.

Human macrophage membranes are electrically excitable (McCann *et al.*, Science, 219:191-193, 1983). A receptor-ionophore model that signals phagocytosis and inflammatory response has been proposed for mouse macrophages where the IgG Fc receptor is described as a ligand-dependent ion channel (Young *et al.*, Nature, 305:186-189, 1983). Voltage-gated potassium channels recently identified in human T-lymphocytes have been associated with the mitogenesis that occurs with their proliferation as part of the immune response (De Coursey *et al.*, Nature, 307:465-468, 1984). These observations suggest that membrane receptors and ion transport processes play a significant and mechanistic role during activation of leukocytes. We are now seeking correlations between macrophage conductance systems and activities such as phagocytosis, secretion, antigen recognition, and receptor-ligand interactions.

Measurements of single channel currents in human macrophages and in a human monocytoid cell line (U937) reveal several types of channels: (1) Rapid, flicker-like channels with brief open times (5-15 msec) and uniform amplitudes at a given voltage that are about twice as large as types 2 and 3. Their frequency of occurrence is voltage dependent. (2) Longer-lived (50-200 msec) whose frequency of opening is also voltage dependent. Amplitudes are uniform at a given holding voltage. (3) Very long-lived channels that remain open from about 400 msec to 2 seconds or more are uniform in amplitude at a given voltage but are not voltage dependent. These channels are less frequently seen than types 1 and 2. (4) The presence of other types is suggested by the occasional occurrence of channels with significantly different amplitudes from the other channel types at a given voltage.

Ion exchange experiments are in progress to identify and further characterize these channels.

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256.17 INWARD RECTIFYING POTASSIUM CURRENTS RECORDED FROM RAT BASOPHILIC LEUKEMIA CELLS BY WHOLE CELL PATCH CLAMP.
Stephen R. Ikeda and Forrest F. Weight. Laboratory of Preclinical Studies, National Institue on Alcohol Abuse and Alcholism, Rockville, MD 20852.

The rat basophilic leukemia cell line (RBL-2H3) has been used as a model system for the biochemical study of excitation-secretion coupling and immediate hypersensitivty reactions. Recent studies provide evidence that immunoglobulin E (IgE) mediated histamine and serotonin release from RBL-2H3s is associated with a membrane depolarization as determined by radioactive probes (Sagi-Eisenberg, R. and Pecht, I., J. Membrane Biol. 75:97, 1983; Kanner, B. and Metzger, H., PNAS 80:5744, 1983). In this study we have characterized the electrical properties of RBL-2H3 cells using the whole cell patch clamp technique.

Rat basophilic leukemia cells were grown in minimal essential media (MEM) supplemented with 20% fetal calf serum at 37°C in a humidified 10% CO_2 atmosphere. All recordings were done at 31-35°C. Resting membrane potential in normal physiological solution was about -70 mV. Cell input resistance, measured between -20 to +50 mV, ranged from 1.3-21.2 Gohms. Voltage clamped cells were held at -70 mV and given command pulses from -150 mV to +50 mV. Hyperpolarizing pulses elicted a rapid voltage-dependent inward current which was accelerated with increased hyperpolarization. In addition, at very hyperpolarized potentials (-120 to -150 mV), there was often a prominent "sag" in the current. The inward current appeared to reverse polarity at approx. -70 mV, although it was greatly attenuated at potentials positive to this value. Removal of external K^+ completely abolished the current. Increasing the external K^+ from 5.4 mM to 10 mM increased the amplitude of the current and shifted the reversal potential to more depolarized values. Inclusion of 1.0 mM $BaCl_2$ or CsCl in the bathing solution reversibly blocked the current. Isomolar replacement of external Na^+ with choline reduced the current amplitude and abolished the "sag" seen at hyperpolarized potentials. It is concluded that RBL-2H3 cells possess an inward K^+ current similar to the anomolous rectifier seen in other cells. The role of this current, if any, in excitation-secretion coupling requires furthur studies. (Supported in part by a PRAT fellowship to SRI from NIGMS)

256.18 DIFFERENTIAL EXPRESSION OF TWO POTASSIUM CONDUCTANCES IN THE MACROPHAGE-LIKE CELL LINE, J774.1.
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J774.1 cells, a well-characterized mouse-derived macrophage-like cell line were voltage clamped using whole-cell patch-clamp techniques. Patch electrodes contained 145 mM Kcl, 10 mM NaCl, 1.0 mM $MgCl_2$, 0.1 mM $CaCl_2$, 1.1 mM EGTA and 10 mM HEPES, pH 7.3, and had resistances of 2-6 Mohms. Cells were maintained in spinner culture and were plated onto tissue culture dishes at various times before recordings were done. Cells recorded from 15 min to 72 hr postplating (PP) had resting membrane potentials ranging from -66 to -84 mV. I-V curves indicated that cells sometimes exhibited inward and/or outward rectification. The inward rectification was enhanced by increasing $(K)_o$, inhibited by barium, showed a time-dependent inactivation for steps beyond -120 mV and we believe reflects an inward rectifying potassium conductance similar to that previously described in cultured mouse macrophages (Gallin & Livengood, Am. J. Physiol. 241:C9, 1981). The outward current activated at voltages positive to -40 mV showed time-dependent inactivation and had a reversal potential determined from tail currents of -80 mV, indicating it was a potassium conductance. A similar outward potassium conductance recently has been described in mouse macrophages (Ypey and Clapman, PNAS, 1984). The inactivation of each of these conductances was evident in the prominent hysteresis present in I-V curves obtained with slow voltage ramps. The expression of both inward and outward potassium conductances varied with time after plating. Up to 2 hr PP cells exhibited no inward rectification and little outward rectification. Eighty percent of cells 2 to 6 hr PP showed prominent inactivating outward currents, while only 10% of these cells exhibited inward rectification. After 7 hr or longer PP, 90% of the cells exhibited prominent inward rectification while the number of cells expressing inactivating outward currents decreased, reaching zero at 24 hr. These findings indicate that J774.1 cells exhibit two different voltage-dependent potassium conductances whose expression varies with time following adherence. It is known that adherence changes the functional properties of macrophages and it is possible that the presence or absence of these conductances relate to these changes.

256.PO **GABA INCREASES CALCIUM ACTION POTENTIAL DURATION IN LAMPREY PRIMARY MECHANOSENSORY NEURONS.** J.P. Leonard and W.O. Wickelgren. Dept. Physiology Univ. Colorado Sch. Med. Denver, CO 80262.

In the presence of TTX and potassium channel blockers (tetraethylammonium and/or diaminopyridine) the normal Na action potential in primary mechanosensory neurons is replaced by a Ca action potential (CaAP) 20 to 2000 ms in duration followed by a hyperpolarizing afterpotential ($t_{1/2}$ 100-3000 ms) due to a calcium-activated K conductance (gK_{Ca}). Bath application of GABA had no effect on the Na action potential but caused a dose-dependent ($5x10^{-5}$M to 10^{-2}M) increase in the CaAP duration (11-63%). GABA had no effect on the resting potential or the passive I-V characteristics of the cell, but during the plateau phase of the CaAP, GABA produced a decrease in input conductance, suggesting that its action is to block a conductance activated during the CaAP. GABA had no effect on the normal Na action potential even when it was much prolonged by the addition of K channel blockers along with 2 mM Cd to prevent the CaAP. This suggests that GABA does not act on a voltage-dependent conductance but rather acts on a conductance that is activated by Ca entry into the cell, possibly gK_{Ca}. If the action of GABA is to partially block gK_{Ca}, then it should have no effect on barium APs, since barium entry in these cells, as in other neurons, does not activate a gK. In agreement with this, GABA had no effect on the 4 to 40 sec action potentials in these neurons produced when Ba was substituted for Ca. Further, in the absence of GABA the duration of the hyperpolarizing afterpotential which follows the CaAP is positively correlated with the duration of the CaAP. However, on addition of GABA the hyperpolarizing afterpotential is not increased even though the CaAP duration is increased. We tentatively conclude that GABA is partially blocking the gK_{Ca} in these cells.

The pharmacology of the GABA effect was investigated. The $GABA_A$ receptor antagonists (bicuculline-100 uM, picrotoxin-100 uM, and curare-500 uM) did not block GABA's effect. The $GABA_A$ receptor agonist muscimol (300 uM) and the $GABA_B$ receptor agonist baclofen (1-10 mM) had inconsistent effects on the CaAP. These results suggest that the effect of GABA on the CaAP in these cells may be mediated by yet a third type of GABA receptor. Other potential neurotransmitters tested did not alter the CaAP : norepinephrine (1 mM), acetylcholine (carbachol, 1 mM), and met-enkephalin (10 uM). Supported by NIH NS-07083.

ACTION POTENTIALS AND ION CHANNELS V

257.1 VOLTAGE CLAMP ANALYSIS OF AN INWARDLY RECTIFYING IONIC CURRENT IN NEURONS FROM CAT SENSORIMOTOR CORTEX. W.J. Spain*, P.C. Schwindt, C.E. Stafstrom, and W.E. Crill. V.A. Hospital, Seattle, WA 98108, and Depts. of Physiology and Biophysics, and Medicine (Neurology), University of Washington School of Medicine, Seattle, WA 98195.

Neurons from layer V of cat sensorimotor cortex were analyzed in an in vitro slice preparation using single microelectrode voltage- and current-clamp techniques. Constant current pulses in either the hyperpolarizing or subthreshold, depolarizing direction cause the membrane potential (Em) to attain an early peak and decay (sag) to a steady level. Upon termination of the pulse, Em transiently overshoots resting potential (RP) in the opposite direction. The voltage sag seen upon both depolarization and hyperpolarization is caused by a slow, inward, ionic current (anomalous rectifier - I_{AR}) that is on at RP. I_{AR} becomes increasingly activated by hyperpolarization and deactivated by depolarization from RP. Instantaneous I-V curves from various holding potentials were linear from +10mV to -40mV relative to RP. Estimated reversals of I_{AR} were 20-50mV positive to RP in different cells. Increasing extracellular K^+ concentration caused depolarization, increased slope and amplitude of the steady I-V curve negative to RP, and a marked increase in I_{AR} chord conductance (G_{AR}) at each potential measured. Conversely, substitution of TRIS for extracellular Na^+ caused hyperpolarization, decreased slope and amplitude of the steady I-V curve, and a decrease in G_{AR} at each potential. I_{AR} is not affected by TTX (10^{-6} M), TEA (20mM), 4-AP (2mM) or substitution of Co^{++} or Ba^{++} for Ca^{++} in the perfusate. Perfusate containing CsCl (2mM) abolished I_{AR} (both positive and negative to the spike AHP reversal potential which was used to estimate Ek) and resulted in hyperpolarization. Our data indicate that I_{AR} flows through a conductance that is increasingly activated with polarization from at least 20mV positive to RP to about 30mV negative to RP. The blocking effect of Cs^+ suggests that I_{AR} results from the passage of both Na^+ and K^+ through a single type of channel which is resistant to a variety of other standard blocking agents. The alteration of G_{AR} by altered extracellular Na^+ and K^+ and by addition of Cs^+ indicate control of the effective channel conductance by these (and possibly other) cations. The presence of I_{AR} both positive and negative to RP indicates that I_{AR} plays a role in establishing RP as well as influencing subthreshold Em behavior, e.g., during the AHP and in the interspike interval during repetitive firing. These properties are most similar to the inward rectifier described in cultured dorsal root ganglion cells (Meyer and Westbrook, 1983). Supported by the Veterans Administration and NIH grants NS16792 and GM07266.

257.2 NEOCORTICAL NEURON AFTERHYPERPOLARIZATION: MULTIPLE FORMS AND ANOMALOUS RESPONSE TO MEMBRANE POLARIZATION. P.C. Schwindt, W.J. Spain*, C.E. Stafstrom, M.C. Chubb*, and W.E. Crill. V.A. Hospital, Seattle, WA 98108, and Depts. of Physiology and Biophysics, and Medicine (Neurology), University of Washington, Seattle, WA 98195.

The afterhyperpolarization (AHP) of cat layer V neocortical neurons studied in an in vitro slice can take several forms depending on stimulus conditions and often shows an anomalous response to membrane polarization. Three AHP components were studied in detail. These include an early, negative peak occuring immediately after a spike evoked from rest (AHP_S), a slower, longer component (AHP_I: 100-200ms duration) which develops after several spikes, and a very prolonged component (AHP_L: 1-5 sec duration) seen only after firing for ca. 100Hz for >200 ms. The amplitude or reversal potential (when it can be measured) of each AHP type becomes more positive as $[K^+]_o$ is raised, but other conductance components also strongly influence AHP form and behavior. AHP_S is easily reversed (5-15mV > RP) by membrane polarization, but reverses positive to rest in some cells. AHP_S is replaced by a prominent delayed depolarization (DD) when Co^{++} is substituted for Co^{++} or TEA added to the perfusate. Apparently, AHP_S amplitude and reversal are influenced by the depolarizing ionic currents responsible for the DD. Following several spikes, AHP_S disappears and is replaced by AHP_I. Following more spikes at an adequate rate, AHP_L appears. Although of small amplitude (ca. 4mV) following a single "burst" of spikes, the slow time course of AHP_L allows it to "summate" to 15-20mV > RP following repetitive bursts and to decay over many seconds. Both AHP_L and AHP_I are rarely reversed by polarization. When it is observed, reversal occurs ca. 30 mV negative to RP, far negative to AHP_S reversal. A null potential or even increased AHP amplitude upon polarization is more common. This behavior is caused in part by activation of the anomalous rectifier I_{AR} upon hyperpolarization (see Spain et al, this session). In spite of its long time course, AHP_L appears due, in part, to a conductance increase; e.g., besides showing occasional reversal, AHP_L is seen in the presence of TTX following depolarization to potentials which activate an outward current of equivalent time course during voltage clamp. Several of the slow AHP properties could be explained by a predominately dendritic location of the relevant K^+ conductance.

Supported by the Veterans Administration and NIH grants NS16792, NS06408 and GM07266.

257.3 SINGLE ELECTRODE VOLTAGE CLAMP OF THE SLOW AHP CURRENT IN RAT HIPPOCAMPAL PYRAMIDAL CELLS. B. Lancaster and P.R. Adams. Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

We have examined some properties of the outward current responsible for the slow afterhyperpolarization (AHP) in hippocampal pyramidal cells using a single electrode voltage clamp (SEVC). In the presence of 1μM TTX, depolarizing current pulses (10-100 msec duration, usually 2 nA amplitude) normally evoked AHPs. If the voltage clamp was activated at the end of the depolarization, the membrane current underlying the AHP could be observed. As expected (Hotson and Prince, J. Neurophysiol. 43, 409) this current was dependent on both Ca^{2+} and K^+. It was reversibly abolished by 200 μM Cd^{++}, and enhanced by adding TEA (5mM), when frank calcium spikes were evident during the depolarizing pulse. It displayed an approximately 20-25 mV shift in reversal potential for a 3-fold change in external K^+ concentration, although outward rectification in normal (2.5 mM) K^+ hampered accurate determination of reversal. The current was also reduced or blocked by 2-4 μM norepinephrine (Madison and Nicoll, Nature 299, 636). It thus displays the properties expected of a current underlying the AHP. The current had a distinct rising phase with a peak (amplitude typically 100-300 pA at rest potential) at 500-700 msec at 27°C. This rising phase was not observed if the voltage clamp was activated with a 1 second delay following the end of the current pulse, and so was not an artefact of activation of the SEVC. The rising phase was also observed when the current was reversed in high K^+, and is thus unlikely to be due to a superimposed inward current. Within the range of membrane potentials that could be reasonably clamped (-50 to -100 mV) the decay of the outward current was largely voltage insensitive. The relationship between the I_{AHP} evoked using this hybrid current/voltage clamp protocol and the outward currents evoked by depolarizing steps under single voltage clamp is being investigated. The slow AHP current recorded in these experiments resembles I_{AHP} of bullfrog ganglion cells (see abstracts in this volume) rather than I_C. Supported by NS 18579 and the Klingenstein Fund. B.L. acknowledges receipt of a travel grant from the Wellcome Trust (U.K.).

257.4 MODELLING OF CA^{2+} CONCENTRATION CHANGES EVOKED BY ACTIVITY IN CAT SPINAL MOTONEURONS. K. Krnjević[1], M. Morris and J.F. MacDonald, [1]Dept. Anaesthesia Research, McGill University, Montreal, and Dept. Pharmacology, University of Toronto, Toronto, Canada.

As already reported (Krnjević et al., 1983, Can. J. Physiol. Pharmac. 61, Axiii) antidromic or direct stimulation at 10-20 Hz for 30-60 s evokes a small but reproducible increase in intramotoneuronal $[Ca^{2+}]$ (measured with Ca-sensitive microelectrodes), on the average amounting to 10-20% above a mean basal level of just under 1 μM.

An attempt was made to fit these findings in the context of previous information on Ca^{2+} currents in cat motoneurons: according to Barrett & Crill (J. Physiol. 1980, 304, 231) a slow, probably Ca-mediated current has a peak intensity about 1/10 of the peak Na current. Assuming therefore that Ca^{2+} influx accounts for 1/10 of the motoneuronal action potential and taking the membrane capacitance as 2.5 μF. $cm^{-2}s^{-1}$, the charge displacement due to Ca^{2+} flux is 18 nC. cm^{-2} per spike, or 180 $nC.cm^{-2}s^{-1}$ during 10 Hz stimulation - which is equivalent to a Ca^{2+} influx of 900 $fmol.cm.^{-2}s^{-1}$.

In a spherical motoneuron with a radius of 35 μm, the increase in mean $[Ca^{2+}]_i$ caused by 10 Hz stimulation for 30 s would therefore be 23 μM. The mean increase in $[Ca]_i$ actually observed was 90 nM, that is only 1/250 of the above. Evidently most of the inflowing Ca^{2+} is rapidly sequestered, leaving only 1/250th free. If Ca^{2+}-binding is proportional to $[Ca]_i$ and is rapidly reversible, the effective diffusion coefficient would be reduced by the same factor of 250. This would permit the build-up of substantial gradients of $[Ca]_i$ between the submembrane region and the centre of the cell, and also slow down correspondingly the kinetics of intracellular $\Delta[Ca^{2+}]$.

If we therefore take $D = 4 \times 10^{-8}$ $cm.^2$ s^{-1}, and further assume that Ca^{2+} is transported out of the motoneuron at a rate (K) proportional to $[Ca^{2+}]_i$, the mean observed half-time of decay of $\Delta[Ca]$ (23 s) would require that K be no less than twice the value of K observed in the squid axon. Thus, the salient feature of Ca^{2+} influx in motoneurons appears to be a highly efficient and rapid sequestration of free Ca^{2+} (99%): if this is taken into account, the observed changes in $[Ca]_i$ resulting from repetitive stimulation are reasonably consistent with a simple spherical model of diffusion and transport.

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257.5 SLOW INWARD CURRENTS AND THEIR ACTIVE POTENTIALS DECAY WITH REPETITIVE STIMULATION. J.F. MacDonald and J.H. Schneiderman. Playfair Neuroscience Unit, Toronto Western and Wellesley Hosp., Univ. Toronto, Ont. M5T 2S8.

Experiments were performed upon mouse spinal cord neurons grown dissociated in tissue culture by conventional techniques. In the presence of elevated Ca (5 mM), TTX and TEA (25 mM) depolarizing pulses passed across the membrane evoked a series of active potentials. These included a "fast" spike (2 to 50 ms) which peaked at values more depolarized than 0 mV. Subsequent to this a "slow" action potential was observed (-10 to -20 mV peak, duration 100 ms to 2000ms). The "slow" action potential merged with "after-depolarizations" which were graded in amplitude and duration.

Repetitive stimulation at frequencies as low as 0.02/s demonstrated that each of the above described potentials underwent a decrement of duration and amplitude which was fully reversible. Microperfusion of Cd blocked or depressed all of these potentials suggesting a primary calcium-dependency. The organic calcium antagonists verapamil and nifedipine were also capable of reducing these active potentials although their actions were not as specific as Cd. Furthermore, microperfusion of Ba greatly prolonged these potentials but did not prevent the frequency-dependent decay. Instead it appeared to be facilitated. Similar results were observed when EGTA was permitted to diffuse from the intracellular recording electrode.

Two-electrode voltage-clamp demonstrated that a variety of inward and outward currents were activated by depolarizing voltage-steps including a "slow" inward current, a "slow outward" current and a "fast" inward current. When voltage-steps were repeated at regular intervals the "slow" inward current also demonstrated a reversible decay. These results suggest that voltage-dependent calcium currents in cultured spinal cord neurones undergo some form of slow "inactivation".

257.6 SODIUM VALPROATE SELECTIVELY LIMITS SUSTAINED HIGH FREQUENCY REPETITIVE FIRING OF CULTURED MOUSE NEURONS. D.M. Rock*, M.J. McLean* and R.L. Macdonald. Dept. of Neurology, Univ. of Michigan, 1103 E. Huron, Ann Arbor, MI 48104.

The effect of the anticonvulsant drug, sodium valproate, on sustained high frequency repetitive firing (RF) of action potentials and iontophoretic GABA responses was studied.

Mouse spinal cord and cortical neurons were maintained in primary dissociated cell culture for 4-6 wks prior to experiments. For experiments, the cell cultures were bathed in protein-free phosphate buffer with elevated magnesium (10 mM) to abolish spontaneous activity. Cells were impaled with high resistance (20-50 MΩ) glass microelectrodes filled with either 4 M potassium acetate or 3 M potassium chloride to record transmembrane potentials. In control solutions, progressively greater depolarizing pulses evoked sustained trains of action potentials firing at increasing frequencies. Sodium valproate was then introduced by adding aliquots of concentrated stock solution to the bath. In iontophoretic experiments, GABA (0.5 M, pH 3.2) was ejected by passing positive rectangular current pulses (5-20 nA, 400 msec) through a pipette positioned ∿ 2 μm from the impaled cell. Then sodium valproate was introduced by pressure ejection from a blunt tipped (5-10 μm) glass micropipette. The range of drug concentrations studied included values equivalent to cerebrospinal fluid concentrations of patients with therapeutic serum levels (2-30 μg/ml).

Sodium valproate limited RF to a few action potentials in a concentration-dependent manner. The effect commenced at 0.25 μg/ml and was maximal at 2.0 μg/ml. Sodium valproate had no effect on postsynaptic responses to iontophoretically applied GABA in most neurons, but produced small enhancements (10-30%) in ∿ 20% of cells only at higher concentrations (10-140 μg/ml).

Limitation of high frequency repetitive firing of action potentials may be an anticonvulsant mechanism of action for sodium valproate.

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257.7 PHENYTOIN AND CARBAMAZEPINE LIMIT SUSTAINED HIGH FREQUENCY REPETITIVE FIRING OF ACTION POTENTIALS OF HIPPOCAMPAL NEURONS IN CELL CULTURE AND TISSUE SLICES. M.J. McLean*, C.P. Taylor and R.L. Macdonald, Dept. of Neurology, Univ. of Michigan and Parke-Davis Research Lab., Ann Arbor, MI 48109

The anticonvulsants phenytoin (PT) and carbamazepine (CBZ) limit sustained high frequency repetitive firing (RF) of spinal cord and neocortical neurons in cell culture at concentrations equivalent to therapeutic plasma free (unbound to albumin) levels (1-2 μg/ml and 0.8-2.5 μg/ml, respectively)(McLean and Macdonald, 1983). We have studied the effect of PT and CBZ on RF of hippocampal neurons in cell culture and in vitro slice.

Primary dissociated cell cultures were prepared using hippocampi from 18-20 day (gestational) fetal mice. Intracellular recordings were made in protein-free phosphate-buffer (pH 7.35-7.40) at 37-38°C. All neurons spontaneously fired action potentials, and about half demonstrated bursts with depolarizing shifts. About 60% of neurons fired sustained trains of action potentials during long (450 msec) depolarizing current pulses. However, in the presence of the calcium channel blocker verapamil (1 μg/ml) all neurons fired in sustained fashion and spontaneous bursting persisted. Addition of PT (2 μg/ml) or CBZ (2-2.5 μg/ml) limited RF to a few action potentials without inhibiting bursts.

Hippocampal slices were prepared from adult rats and superfused with artificial CSF at 37°C. CA1 and CA3 neurons fired 6-12 APs during 450 msec depolarizing pulses. During superfusion with PT (2-4 μg/ml)-containing solution, the number of APs was limited to 2-3. Return to PT-free solution restored APs to control numbers. Mossy fiber stimulation produced brief depolarizations with 2-3 APs in both control and PT-containing solution. Thus, no effect of PT on short bursts of APs was seen.

Thus, sustained RF can be demonstrated in cultured neurons from various CNS regions, including hippocampus. RF of cultured hippocampal neurons and of pyramidal neurons in slices is limited by PT and CBZ at "therapeutic" concentrations. Limitation of RF may be an important mechanism whereby PT and CBZ exert anticonvulsant efficacy.

McLean, M.J. and Macdonald, R.L. JPET 227:779-789, 1983.

Supported by NIH Grant R01 NS19692 (RLM) and TIDA NS00817 (MJM).

257.8 EXPRESSION OF INTRINSICALLY GENERATED ELECTRICAL ACTIVITY IN DEVELOPING PURKINJE NEURONS. C.L. Franklin* and D.L. Gruol. (SPON: S. Henriksen), Division of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Vertebrate CNS neurons display characteristic patterns of activity as a result of their unique intrinsic electrical capabilities and specific neuronal circuitry. For several vertebrate CNS neuronal types, including the Purkinje neuron (PN) of the cerebellum, intrinsically generated electrical activity plays a prominent role in defining the final activity pattern. We have selected this neuronal type as a model to examine the ionic mechanisms that generate intrisic activity in vertebrate CNS neurons and to investigate the developmental sequence in the maturation of these mechanisms during neuronal differentiation. To facilitate these studies, a culture preparation of fetal rat cerebellum was used. In initial studies (Franklin and Gruol, Neurosci. Absts, 1983), we were able to establish morphological criteria for identification of developing PNs in culture and to correlate the morphological stages of development in culture with that described for PNs in vivo. In the present experiments, we have used extracellular recording techniques to assess the electrical capabilities of immature PNs displaying morphologies characteristic of the developmental stages. Recordings were obtained from over 100 PNs in 4 to 60 day cultures. Mature cultured PNs (over 20 days in vitro, DIV) commonly display two modes of spontaneous activity thought to arise from intrinsic, voltage-sensitive mechanisms (Gruol, Brain Res., 263, 1983): 1) a regular simple spike (SS) pattern and 2) intermittent burst events similar to complex spikes (CS). At the youngest culture age studied (4 DIV; rounded with perisomatic processes) the majority of PNs were silent or displayed low frequency, irregular SS activity. Glutamate could evoke SS activity in many of the silent PNs. At 6-8 DIV (early apical cone stage), the PNs displayed regular or intermittent patterns of SS activity, similar to (but at a lower frequency than) that observed in mature PNs. At later stages, when dendrites were prominent, both SS and CS patterns were observed. These data are compatible with the view that the ionic mechanisms generating the regular SS pattern are established early in development and are localized to the somal region, whereas the ionic mechanisms generating the CS are established later in development and are localized to the dendritic region. (supported by NIAAA 06420)

257.9 NON-SYNAPTIC FAST DEPOLARIZING POTENTIALS (FDPs) IN RAT SUPRAOPTIC NUCLEUS (SON) NEURONS: FUNCTIONAL ROLE IN PHASIC ACTIVITY. C.W. Bourque and L.P. Renaud. McGill University and the Montreal General Hospital, Montreal, Que. Canada.

SON neurons can respond to osmotic and cardiovascular stimuli with the evolution of phasic discharges, a pattern that is highly efficient at inducing neurohypophyseal hormone release and is therefore thought to represent a functional adaptation of the magnocellular neuroendocrine cell (MNC). Previous intracellular recordings of SON MNCs in vitro have revealed that their action potentials are followed by depolarizing afterpotentials (DAPs). Spikes occurring in rapid succession allow DAPs to summate and establish the depolarizing plateau potential that underlies a phasic (burst) discharge (Andrew & Dudek; Science,221,1983). We now report that action potentials occurring at the onset of a burst may be initiated by fast depolarizing potentials (FDPs) of non-synaptic origin

Thirty-two phasic SON neurons were recorded intracellularly from intravascularly perfused rat hypothalamic explants. In twenty-six cells, burst onset was characterized by a depolarization phase (ca. 1 mV/sec) and the appearance of variable (2-10 mV) FDPs occurring in rapid succession. Within a few seconds, FDPs elicited full blown spikes whose DAPs summated to establish the plateau sustaining the burst. While FDPs could be observed toward the end of a burst, these always disappeared as the plateau repolarized.

Neuronal hyperpolarization abolished all spontaneous bursting activity and demonstrated that FDPs did not represent patterned synaptic inputs. During membrane hyperpolarization, random postsynaptic potentials (PSPs) were seen to increase their amplitude in a voltage dependent manner. Conversely, membrane depolarization from ca. -80 mV revealed a population of depolarizing potentials identical to the FDPs described above. These potentials were evoked in an all or none fashion in a narrow voltage range extending from about 5 mV below the threshold for spike initiation. Alternatively, these potentials could be evoked by any manipulation causing the cell to approach this threshold eg. synaptically evoked EPSPs, burst induced DAPs, anode break depolarizations or direct current injections. In all cases FDPs were resistant to the reversible blockade of synaptic transmission with 15 mM magnesium.

While spontaneous EPSPs may trigger spikes in MNCs, the occurrence of FDPs in rapid succession might be especially significant in causing DAPs to summate and establish the plateau potential. (Supported by the Canadian M.R.C. and F.R.S.Q).

257.10 EVOKED CHARACTERISTICS OF DENDRITIC SPIKES IN CA1 HIPPOCAMPAL PYRAMIDAL CELLS. RW Turner, TL Richardson and JJ Miller, Dept. Physiol, UBC, Vancouver, B.C. Canada V6T 1W5

Intradendritic recordings were carried out in the CA1 region 150u from the stratum pyramidale (SP) in hippocampal slice preparations. An extracellular electrode in SP above the impalement monitored corresponding population spike activity. Stimulating electrodes were placed in stratum radiatum (SR), stratum oriens (SO) or the alveus for ortho- or antidromic activation of CA1 neurons.

SR stimulation evoked an EPSP-IPSP sequence with conductance increasing from the peak of the EPSP to a maximum during the IPSP. At threshold a single spike of 43 ± .9mV amplitude was evoked on the EPSP ($\bar{x}$ ± sem; n=27; measured from the breakpoint), displaying variable latency but arising coincident with or after the falling negative phase of the population spike. This compares with an average somatic spike of 77 ± .7mV (n=9) evoked primarily from a negative notch on the EPSP corresponding to the falling negative phase of the population spike (Brain Res. 294: 255, 1984). Although the dendritic spike exhibited 'all-or-none' characteristics at threshold, there was a variable threshold potential and the spike amplitude was decreased if it discharged on the falling edge of the EPSP. SO stimulation also evoked an EPSP-IPSP sequence and a spike of 55± 2.3mV amplitude (n=15) in the apical dendrite. This spike displayed a similar latency shift and relationship to the population spike as that evoked by SR. Alvear stimulation evoked a graded IPSP associated with a conductance increase and a single antidromic spike of 62 ± 2.2mV amplitude (n=14). This spike displayed a constant latency and was evoked following the peak of the population spike. All dendritic spikes could be blocked by a conditioning antidromic stimulus at C-T intervals which result in an inhibition of the test population spike (5-120 msec). The similarity of apical dendritic spike characteristics evoked by SR, SO and antidromic stimulation together with a variable threshold potential and susceptibility to recurrent inhibition suggests that these spikes may be of somatic rather than dendritic origin.

257.11 AN ELECTROPHYSIOLOGICAL STUDY OF RAT SENSORY NEURONES INFECTED WITH HERPES SIMPLEX VIRUS. M.L. Mayer & M. H. James*. Departments of Pharmacology and Biochemistry, St. George's Hospital Medical School, London, U.K.

Sensory neurones in dissociated cultures prepared from neonatal rat dorsal root ganglia were infected with either of two strains of herpes simplex virus type 1 (HSV 1) isolated from clinical specimens. After a latency of 8-10 hours, during which period the replication of HSV in glial cells and neurones was demonstrable by immunofluorescence, sensory neurones displayed marked changes in electrical activity.

A strain of HSV 1 (03 syn), which causes fusion in MRC-5 cells, consistently induces spontaneous electrical activity in DRG neurones, without altering the resting potential or input resistance. The spontaneous activity is due to e.p.s.p. like events which evoke action potentials on crossing threshold. In individual cells the amplitude of the e.p.s.p.s is highly consistent, as though due to a fixed quantity of depolarizing current. The underlying events recorded under voltage-clamp appear to be due to rapidly rising inward currents with decay time constants briefer than that of the e.p.s.p.s. and the membrane time constant. Cadmium, a calcium channel blocker, did not consistently block spontaneous activity, suggesting that it was of non-synaptic origin. When pairs of infected sensory neurones were impaled it was possible to demonstrate constant latency reciprocal excitatory connections, suggestive of remote electrical coupling. Classical electrical coupling could not be demonstrated with large hyperpolarizing electrotonic potentials. Pretreatment of cultures with 50 μM acyclovir, which specifically inhibits HSV replication, blocked the induction of spontaneous electrical activity by HSV 1-03 syn.

A second, non-syncitial strain of HSV 1 (C5 syn+) did not induce spontaneous activity, but had other striking effects on electrical excitability. Cells infected with HSV 1-C5 syn+ were unable to generate fast sodium dependent action potentials, but always displayed pronounced depolarization activated outward rectification, and in some cases high threshold, slow Ca^{++} like action potentials. Hyperpolarization activated inward rectification which occurs in the majority of sensory neurones was absent in neurones infected with HSV 1 C5 syn+, such that the input resistance (measured using a conventional intracellular electrode) could be as high as 1 GΩ.

257.12 DEVELOPMENT OF ELECTROPHYSIOLOGICAL PROPERTIES OF RAT PERIPHERAL MYELINATED NERVE FIBERS. P.J. Ricot* and M. Rasminsky. Neurosciences Unit, Montreal General Hospital and Departments of Physiology and Neurology & Neurosurgery, McGill University, Montreal, Canada.

The axon membrane of normal mature myelinated fibers is differentiated into nodal regions with a high density of sodium channels and no potassium channels, and internodal and paranodal regions containing potassium channels but few, if any sodium channels (Ritchie & Rogart, P.N.A.S. 74:211,1977; Chiu & Ritchie, J.Physiol. 313:415,1981). We have now studied the conduction properties and spatial distribution of membrane currents in rat spinal root fibers between days 13 and 28 of postnatal development.

From measurements of compound action potentials (CAPs), maximum conduction velocity (CV) was found to increase from 9±2 to 32±13 m/s and from 5±2 to 21±5 m/s for lumbo-sacral dorsal and ventral roots (DRs and VRs) respectively between 13 and 28 days (34°C). Elimination of functional potassium channels in developing nerves was monitored by following the increase in the area of the CAP on exposure to 1mM 4-aminopyridine (4AP). Enhancement of the CAP fell from 258±144 to 52±27% and from 110±37 to 17±15% for DRs and VRs respectively betweeen 13 and 28 days.

In studies of conduction in single VR fibers we found that between 13 and 19 days, internodal length is very variable for a given fiber. Most fibers attain uniform internode length by the end of the fourth post natal week. Internodal conduction time (ICT) decreases monotonically with age. Fibers with CV>40% of maximal CAP CV had ICT which decreased from 77±33 μsec at 13-14 days to 33±7 μ sec at 23-25 days whereas fibers with CV<40% of maximal CAP CV had ICT which decreased from 192±63 μsec at 13-14 days to 54±23 μ sec at 23-25 days (37°C).

Late outward currents identified as potassium currents by blockage with 4AP were seen only at some nodes of any given fiber. Large early outward currents suggestive of increased nodal capacitance and associated with focal increases in ICT were seen at some nodes with no necessary association with the presence of late outward current.

These data suggest: 1) that the rate of functional maturation is different for different fibers; 2) that within a given fiber the maturation of successive nodes of Ranvier does not occur with absolute uniformity; and 3) that factors other than decrease in nodal area are of importance in the elimination of potassium channels from nodes of Ranvier.

257.13 EFFECTS OF POTASSIUM CHANNEL BLOCK ON IMPULSE REPOLARIZATION IN A VERTEBRATE MYELINATED AXON. P.G. Funch, R.A. Stockton, Jr.* and D.S. Faber. Div. of Neurobiology; Dept. of Physiology; SUNY at Buffalo, NY and The Dent Neurologic Institute, Buffalo, NY.

The questions of the existence of voltage-sensitive potassium channels in vertebrate central myelinated axons and their functional role in action potential repolarization have been addressed using the goldfish Mauthner axon. The axon hillock-initial segment spike component of antidromically propagating impulses was eliminated by extracellular ejections of tetrodotoxin within the axon cap. Voltage-sensitive potassium channel blockers (e.g. 4-aminopyridine, tetraethylammonium, Cs^+) were then assessed for their effects on axonal spike waveform when applied either by localized extracellular ejections or by intra-axonal ejections at one or several sites along the axon. Intra-axonal and intra-myelin sheath recordings were made before and after such ejections. The recordings were digitized on-line (142 KHz sampling rate) and the waveforms and their computed derivatives were subsequently analyzed. The isolated axonal impulse has an amplitude of 110 mV from a resting potential of -85 mV, a maximal rate of rise of more than 1300 V/sec, a maximal rate of repolarization of 450 V/sec, a spike width at half maximum amplitude of 220 ± 14 μsec ($\bar{x}$ ± SD), and a maximal undershoot of -1 to -3 mV which occurs 400 μsec after the maximal rate of repolarization.

Following either extra- or intra-axonal application of potassium channel blockers, the undershoot was eliminated and spike width increased up to a maximum of 50-100% greater than control (315 ± 48 μsec). The time course of the development of these phenomena was dependent upon both the drug concentration and the location(s) of the ejection(s). The overall repolarizing phase of the action potential increased in duration and was altered in waveform. The undershoot was replaced by a monotonic repolarization whose amplitude at any specific time was positively correlated with drug dose. However, an undershoot was still observed when recordings were made from within the myelin sheath. The resulting repolarization process may be consistent with passive current flow through axonal and intra-myelin sheath pathways (see Barrett & Barrett, J. Physiol. 323:117, 1982). There was also a a pronounced enhancement of the axon's ability to be repetitively activated in response to a maintained depolarizing current. (Supported by NIH Grant NS17063.)

257.14 TEMPERATURE DEPENDENCE OF ACTION POTENTIALS AND IONIC CURRENTS IN RAT MYELINATED NERVE FIBRES. J.R. Schwarz (SPON: European Neuroscience Association). Inst. of Physiology, UKE, University of Hamburg, D-2000 Hamburg 20, FRG.

There is little information about the action potential and the underlying ionic currents of mammalian myelinated nerve fibres at the normal body temperature and their dependence on temperature (T). Therefore current and potential clamp experiments were performed on 15 single myelinated nerve fibres of the rat (Sprague-Dawley) at 37°C as well as during a change in T between 37° and 0°C. The potassium permeability was blocked by 12 mM TEA added to the superfusing Ringer solution and by internal application of CsCl (by diffusion through the cut internodes).

At 37°C the action potential exhibited a threshold potential of about 20 mV, an amplitude of 114± 3 mV and a duration of 0.3 ms. When T was decreased from 37° to 20°C the action potential duration was prolonged with a Q_{10} = 2.4.

The holding potential, at which 30% of the Na system was inactivated (h_∞ = 0.7), was assumed to be the resting potential (E = -80 mV; Neumcke and Stämpfli, J. Physiol., 329: 163, 1982). The potential dependent activation of the Na permeability, P_{Na}, yielded a maximum P_{Na} = 0.72± 0.1 $cm^3 s^{-1} 10^{-9}$ with a 50% activation at E = -42 mV. When T was decreased e.g. from 20° to 0°C both $P_{Na}(E)$ and $h_\infty(E)$ were shifted by 6 - 9 mV towards more negative membrane potentials.

The effect of a change in T on the membrane parameters was determined in intervals of 0.5 - 1°C. A transition temperature was detected for P_{Na}: from 37° to 10°C it decreased with a Q_{10} = 1.2, at T< 10°C with a Q_{10} = 1.8. The time constants of activation and inactivation of P_{Na} changed with a Q_{10} near 3, tending to larger values at T< 10°C. Na inactivation was described by two exponentials, the amplitude factor of the slower component being 0.2 - 0.3 independent of T.

The large decrease in P_{Na} at T< 10°C and the shift of $h_\infty(E)$ are likely to explain the conduction block at low T in mammalian nerve fibres.

257.15 POTASSIUM CHANNEL BLOCKADE IN SENSORY AND MOTOR FIBERS DURING MATURATION. C. M. Bowe*, J. D. Kocsis and S. G. Waxman. Dept. of Neurology, Stanford University Medical School and Veterans Administration Medical Center, Palo Alto, CA 94304.

Although potassium conductance (g_K) has been shown to have an important role in the repolarization phase of the action potential in a variety of axon systems, its functional significance in mature myelinated fibers has not been demonstrated. Studies on maturing rat sciatic nerve have reported the appearance of a characteristic delayed depolarization and spike burst activity following the application of 4-aminopyridine (4-AP), a potassium channel blocking agent, suggesting that g_K may be important in stabilizing nodal firing properties (Kocsis et al, J. Neurophysiol. 50: 449-463, 1983). Isolated sciatic nerves, ventral and dorsal roots from 4 to 36 week old Wistar rats were studied using a sucrose gap recording chamber. Intra-axonal impalements were obtained with glass microelectrodes filled with 2M KCl. Compound action potentials recorded from sciatic nerves following superfusion with 1 mM 4-AP developed a characteristic late negativity which diminished with increasing age. Ventral root recordings in younger animals (10 weeks) typically showed broadening of the initial negativity with a delayed repolarization. This effect also was reduced during maturation. The application of 4-AP to dorsal roots did not significantly alter the initial negativity but did produce a marked delayed depolarization which persisted during maturation beyond the age when ventral responses were notably reduced. Single axon recordings were obtained from both motor and sensory axons. In 5 week old rats, exposure of ventral root fibers to 4-AP resulted in a broadening of the action potential. In contrast, dorsal root axons, at the same age, gave rise to bursts in action potentials in response to a single stimulus after 4-AP. This bursting behavior is similar to that described in immature sciatic nerve axons. These results indicate a difference in response to g_K blockade between motor and sensory fibers during maturation. This may reflect the presence of pharmacologically and kinetically distinct potassium channels for sensory and motor fibers or, alternatively, differences in the morphophysiological organization of the node or paranode with respect to potassium channel access. Such differences may have important implications for the coding properties of various functional classes of myelinated mammalian axons.
This work was supported in part by the VA, NIH and the National Multiple Sclerosis Society.

257.PO CALCIUM DEPENDENT ELECTRICAL ACTIVITY IN LEECH SALIVARY GLAND CELLS. C.G. Marshall and C.M. Lent. Division of Biology & Medicine, Center for Neural Science, Brown University, Providence, RI 02912.

The salivary glands of both jawed and proboscis bearing leeches comprise unicellular secretory cells which extend individual ductules from each cell body. These exocrine cells are electrically excitable, and fire regenerative, overshooting action potentials of long duration (150 ms) in response to depolarization, and at the cessation of a hyperpolarizing pulse. The anterior salivary glands of the Amazon leech Haementeria ghilianii and the Mexican medicinal leech H. officinalis have provided isolated preparations for electrophysiological studies. Cell diameters range from 200μm to 500μm, and some attain 1mm in the largest individuals.

The action potential is reversibly abolished by 5mM cobalt or manganese, is irreversibly abolished by 1mM lanthanum, and is prolonged then abolished by 2μM methoxyverapamil (D600). The impulse is supported when either strontium or barium replace external calcium, persists in sodium-free solution and is insensitive to 0.1mM tetrodotoxin (TTX). An average increase in overshoot of 25mV results when external calcium is increased from 2 to 20mM, and the action potential is prolonged to 8-10 sec by external application of tetraethylammonium(TEA). The available evidence indicates that the action potential is calcium dependent,and no overshooting impulse can be generated in calcium free solution. While the presence of sodium current has not been positively excluded, it must be of low density if present.

Unlike many other excitable secretory cells, these are not electrically coupled, and so individual cells may be studied without the complication of electrotonic spread of activity in other cells. The cells are robust, and will survive several days in only saline solution. Enzymatic dispersion and maintenance in tissue culture may yield a useful cellular preparation for study of the parametersof stimulus-secretion coupling, particularly since their secretion is accomplished entirely by exocytosis.
Supported by NIH grant NS14882, to C.M.L.

CATECHOLAMINES: BIOCHEMICAL CHARACTERIZATION I

258.1 REGULATION OF TYROSINE HYDROXYLASE ACTIVITY AND mRNA FOR TYROSINE HYDROXYLASE BY CYCLIC AMP AND GLUCOCORTICOIDS IN RAT PHEOCHROMOCYTOMA CELL LINES. A.W. Tank and L.N. Ham*, University of Colorado Health Sciences Center, Denver, Co 80262.

We have isolated a number of subclones of the rat pheochromocytoma PC12 cell line, to be used as model systems for the study of the induction of tyrosine hydroxylase (TH) and mRNA for TH ($mRNA^{TH}$) by cyclic AMP and glucocorticoids. These subclones divide more rapidly (doubling time = approximately one day) than the parent PC12 cells, and TH activity is increased many-fold (2-10 fold depending on the subclone) by either cyclic AMP analogs or glucocorticoids. In these studies TH activity was assayed by the coupled decarboxylase assay (Anal. Biochem. 43, 558, 1971) and $mRNA^{TH}$ was measured using the cDNA clone pTH.4 (J. Biol. Chem. 258, 14, 632, 1983) which contains sequences complementary to the $mRNA^{TH}$.

Basal enzyme activity of TH varies dramatically between the subclonal cell lines. Southern blot analysis of the DNA isolated from subclones possessing either high or low TH activity indicates that the number of genes per cell is approximately equal in the subclones tested. In contrast, when total cellular RNA is isolated from different subclones and $mRNA^{TH}$ measured by RNA dot hybridization or northern blot analysis, only one major RNA species with sequences complementary to the $mRNA^{TH}$ is found in all the subclones tested, and the basal intracellular levels of this RNA species parallel the basal enzyme activities measured in the different subclonal cell lines.

When the subclones are treated with either dexamethasone, a synthetic glucocorticoid, or 8-bromocyclic AMP, both the enzyme activity of TH and the relative intracellular levels of $mRNA^{TH}$ increase. The cyclic AMP-mediated and glucocorticoid-mediated increases in TH activity are approximately equal to the increases in $mRNA^{TH}$ elicited by these inducing agents in each of the subclones tested. In contrast, when the parent PC12 cells are treated with 8-bromocyclic AMP, neither the activity of TH, nor the levels of $mRNA^{TH}$ increase. Treatment of PC12 cells with dexamethasone is associated with a 1.6-2-fold increase in both enzyme activity and $mRNA^{TH}$ levels.

We conclude from these studies that the regulation of the intracellular level of TH in rat pheochromocytoma cells is primarily a consequence of changes in the intracellular level of $mRNA^{TH}$. Supported by USPHS grant NS19749.

258.2 TYROSINE HYDROXYLASE IS PHOSPHORYLATED AT MULTIPLE SITES IN RAT PHEOCHROMOCYTOMA PC12 CELLS TREATED WITH 56 mM K^+. N. Yanagihara*, A.W. Tank, W. Mosimann*, E. Tachikawa* and N. Weiner. Dept. Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

In previous studies we have observed a K^+-depolarization-induced increase in the phosphorylation of two distinct phosphopeptide bands derived from rat pheochromocytoma PC12 cell tyrosine hydroxylase (TH) after trypsin digestion of the enzyme and separation of the phosphopeptides by one-dimensional paper electrophoresis. (Soc. Neuroscience Abstr. 9, 1125, 1983). The phosphorylation of only one of these phosphopeptide bands is enhanced when PC12 cells are incubated with 2 mM dibutyryl cyclic AMP. In the studies presented here, we report the further resolution of these phosphopeptide bands generated by tryptic digestion of TH.

PC12 cells were incubated in the presence of ^{32}P-phosphate (0.5 mCi/ml) for 30 minutes to label intracellular ATP. Cells were then incubated in the presence of 4.5 mM or 56 mM K^+ for five minutes. TH was then isolated by immunoprecipitation, and subjected to SDS-polyacrylamide gel electrophoresis. The ^{32}P-labelled protein band corresponding to the M_r = 60,000 subunit of TH was eluted from the gel and subjected to proteolytic digestion using trypsin for 12-36 hr at 37°C. The generated ^{32}P-phosphopeptides were then separated either by high pressure liquid chromatography (HPLC) on a Lichrosorb RP-18 (10 μm) column using a 0-20% propanol gradient for elution, or by two-dimensional thin layer cellulose analysis using electrophoresis at pH 1.5 in one dimension, followed by ascending chromatography in a sec-butanol: n-propanol: isoamylalcohol: pyridine: H_2O (1:1:1:3:3) solvent system in the second dimension.

Using HPLC we have separated three distinct ^{32}P-phosphopeptide peaks derived from TH. The phosphorylation of each of these peaks increases after high K^+-depolarization of the PC12 cells. One of these peaks is identical to the ^{32}P-phosphopeptide peak isolated from the tryptic digestion of purified TH phosphorylated in vitro by cyclic AMP-dependent protein kinase. Using two-dimensional thin layer analysis we have separated six ^{32}P-phosphopeptides derived from TH. The phosphorylation of at least three of these peptides increases dramatically after high K^+ depolarization of the PC12 cells.

We conclude that TH is phosphorylated at multiple sites in PC12 cells treated with 56 mM K^+. The phosphorylation of these sites is presumably catalyzed by cyclic AMP-dependent and cyclic AMP-independent protein kinases in the PC12 cells. Supported by USPHS grants NS07927, NS09199 and AG03932.

258.3 TYROSINE HYDROXYLASE: PHOSPHORYLATION BY CALMODULIN-DEPENDENT MULTI-PROTEIN KINASE. P. R. Vulliet, J. R. Woodgett and P. Cohen. Medical Science Inst., University of Dundee, Dundee DD1-4HN, Scotland.

Tyrosine hydroxylase purified from rat pheochromocytoma was phosphorylated stoichiometrically by either cyclic AMP-dependent protein kinase or calmodulin-dependent multi-protein kinase from skeletal muscle, but not by five other kinases tested. The activity of tyrosine hydroxylase as assayed by the CO_2 release method was elevated three-fold by cyclic AMP-dependent protein kinase, but no activation was observed after phosphorylation by calmodulin-dependent multi-protein kinase. Phosphorylation produced by cyclic AMP-dependent protein kinase and calmodulin-dependent multi-protein kinase was additive, suggesting different sites of phosphorylation. This was confirmed by analysis of the tryptic phosphopeptides by HPLC and isoelectric focusing which demonstrated that the major sites phosphoryated by each kinase were distinct. Evidence for three distinct phosphorylation sites on tyrosine hydroxylase will be presented.

Calmodulin-dependent multi-protein kinase activity was partially purified from rat pheochromocytoma utilizing a procedure developed for purification of this enzyme from skeletal muscle. The kinase activity exhibited identical properties and substrate specificity to the enzyme isolated from skeletal muscle. The possibility that this enzyme is involved in the regulation of tyrosine hydroxylase activity in adrenergic tissue will be discussed.

This research was supported by NSF grant BNS 8118957 and a travel grant from the Burroughs-Welcome Foundation.

258.4 BOTH PHOSPHAITIDYLSERINE AND CALMODULIN MODULATE CALCIUM-DEPENDENT PHOSPHORLATION OF TYROSINE HYDROXYLASE FROM BOVINE ADRENAL CHROMAFFIN CELLS. R.J. George and J.C. Waymire. Dept. of Neurobiology and Anatomy, Univ of Texas Medical School, Houston, Texas 77025

We have shown previously that tyrosine hydroxylase (TH) in bovine adrenal chromaffin cells undergoes an acetylcholine-stimulated, Ca^{++}-dependent phosphorylation in situ, and that this phosphorylation involves an increase in phosphate at two sites on each TH sub-unit. (J. Bio Chem 257:13699, 1982). Further, under in vitro phosphorylation conditions, Ca^{++} addition to the 100,000 x g supernatant of the chromaffin cells produces a Ca^{++}-dependent phosphorylation of TH which resembles the phosphorylation produced in vitro by acetylcholine (in press). We now report that this Ca^{++}-dependent in vitro phosphorylation of TH can be modulated by either phosphatidylserine or calmodulin.

To investigate the requirements for Ca^{++} stimulated phosphorylation of TH, a 100,000 x g supernatant was subjected to DEAE cellulose chromatography to remove both the calmodulin and phosphatidylserine. The 0.2M NaCl eluate was tested for the presence of Ca^{++}-dependent protein kinase activity which could phosphorylate TH. In the presence of Ca^{++} alone, very little ^{32}P incorporation into TH is observed. Upon addition of either phosphatidylserine or calmodulin, a large Ca^{++}-dependent phosphorylation of the enzyme takes place. Both the phosphatidylserine and calmodulin stimulated reactions require micromolar Ca^{++} and are maximal in the presence of 100 ug/ml phosphatidylserine and 0.7uM calmodulin respectively. Tryptic digests of TH phosphorylated by the two protein kinase activities reveal that both stimulated the incorporation of phosphate into two peptide fragments. However, the pattern of phosphate incorporation produced by the two kinase activities is different. The phosphatidylserine stimulated activity phosphorylates the more electrophoretically mobile peptide to a far greater degree than the more chromatographically mobile peptide. In contrast the calmodulin stimulated activity enhances the incorporation of phosphate into the two peptides equally. In that the calmodulin stimulated protein kinase activity mimics the pattern of phosphate incorporation observed in the acetylcholine stimulated phosphorylation of TH, the calmodulin stimulated kinase, alone, could account for the in situ phosphorylation of TH.

Supported by NS 10061 from NINCDS

258.5 STIMULATION OF PROTEIN KINASE C BY PHORBOL ESTERS INCREASES SYNTHESIS OF CATECHOLAMINES AND PHOSPHORYLATION OF TYROSINE HYDROXYLASE IN SITU. J.P. Johnston and J.C. Waymire. Dept. of Neurobiology and Anatomy, Univ of Texas Medical School at Houston, Houston, Texas, 77030.

Tyrosine hydroxylase, the rate limiting enzyme in the catecholamine synthetic pathway, is thought to be regulated via a phosphorylation-dephosphorylation reaction. Previous results from this laboratory have indicated that the actions of acetylcholine on tyrosine hydroxylase activity in cultured adrenal chromaffin cells may be mediated by a calcium activated, phospholipid dependant protein kinase (Protein Kinase C), and that multiple kinase activity may be involved in the short term regulation of catecholamine biosynthesis (Haycock, et.al., J Biol Chem 257:13699, 1982). In the present studies, we have extended these observations by examining the effects of different phorbol esters, which are known activators of protein kinase C (Castagna, et. al., J Biol Chem 257:7847, 1982.), on the synthesis of catecholamines, and the phosphorylation of tyrosine hydroxyalse in situ.

Cultured bovine adrenal chromaffin cells were stimulated with the following phorbol esters, in the presence of ^{14}C-tyrosine and $^{32}P_i$; 4β-Phorbol 12β-Myristate 13α-Acetate(TPA) Phorbol-12,13-dibutyrate, Phorbol-12,13-dibenzoate, and 4α-Phorbol-12,13-didecanoate. Cells were stimulated for 6 mins and catecholamine synthesis was quantitated by measuring the evolvement of $^{14}CO_2$ during the conversion of ^{14}C-tyrosine to dopamine. Phosphorylation of tyrosine hydroxylase during phorbol stimulation was determined by measuring $^{32}P_i$ incorporation into a 60K protein separated by SDS-PAGE.

TPA, phorbol-12,13-dibenzoate, and phorbol-12,13-dibutyrate stimulated catecholamine synthesis in a dose dependant manner, with a 3 fold maximal increase at 1000 ng/ml. In addition, 4α-phorbol-12,13-didecanoate, which is inactive in stimulating protein kinase C, was without effect in this system. It was also observed that TPA caused a dose dependant increase in the phosphorylation of tyrosine hydroxylase, in a manner similar to that observed for synthesis.

The results of these experiments indicate that stimulation of protein kinase C activity increases both the phosphorylation of tyrosine hydroxylase, and the synthesis of catecholamines. This data lends further support to the hypothesis that protein kinase C may play a role in the regulation of catecholamine synthesis in vivo.

Supported by NS 11061

258.6 DOPAMINE ß-HYDROXYLASE: SUBUNIT CHARACTERIZATION. D.L. Wong, M.K. Speedie* and R.D. Ciaranello. Dept. of Psychiatry, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Conventionally purified bovine dopamine ß-hydroxylase (DBH) appears as a single band on sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis using Coomassie blue staining. However, high resolution silver staining of these same preparations, which allows the detection of nanogram levels of protein, shows that there are a number of minor contaminants copurifying with DBH. To purify DBH to homogeneity, we have employed the following scheme: Chromaffin granules isolated from bovine adrenal medulla by sucrose density sedimentation are lyzed to release soluble DBH. The enzyme is then purified by passage through two molecular sieving columns, Sephadex G-200 and Sepharose 4B. This is followed by affinity chromatography on Concanavalin A-sepharose 4B and finally, by gradient elution on an HPLC anion exchange column. SDS-polyacrylamide gel electrophoresis shows that enzyme so purified consists of three monomeric forms. These monomers can interconverted and finally reduced to two less glycosylated forms using various endo- and exoglycosidases. By varying conditions of deglycosylation, we see a predominance of the lower molecular form of "deglycosylated" DBH. However, at present, it is unclear whether the residual doublet represents two distinct polypeptides or polypeptides with residual glycosylation which can be further reduced to a single protein. Further attempts are being made to separate and characterize the subunits by HPLC, isoelectric focusing, proteolytic mapping, sequencing and amino acid content analysis.

258.7 KINETIC ANALYSIS OF THE UPTAKE OF NOREPINEPHRINE, DOPAMINE AND SEROTONIN BY "CRUDE" AND "PURIFIED" SYNAPTOSOMES OBTAINED FROM DISCRETE REGIONS OF THE RAT BRAIN. R.P. Shank, C.R. Schneider* and W.J. Baldy*. (SPON: A.R. Freeman) Dept. of Biol. Res., McNeil Pharmaceutical, Spring House, PA.

Many studies have been reported during the past 20 years in which K_m and V_{max} values for the uptake of norepinephrine (NE), dopamine (DA), and sertonin (5-HT) by synaptosomal preparations were determined. In many of the studies the biological material was a "crude" synaptosomal preparation (Whittaker P2 fraction), and usually the substrate concentration range was limited to less than a 100-fold difference between lowest and highest concentrations. In order to obtain more precise determinations of the K_m and V_{max} values for the uptake of NE, DA and 5-HT, we have performed a kinetic analysis of uptake using P2 fractions and more purified synaptosomes obtained from discrete regions of the rat brain. Twenty-four substrate concentrations ranging from 1 nM to 1 mM were used and the data were analyzed with the aid of the Pennzyme computer program (Kohn, et al., Comput. Biomed. Res. 12, 461, 1979), which performs a weighted non-linear regression analysis of the untransformed data. This analysis made possible an accurate correction for nonsaturable accumulation of substrate, and provided a best-fit to theoretical curves containing 1 to 4 saturable transport systems. Ascorbate (0.1 mM) and nialimide (0.01 mM) were usually included in the incubation medium, but preliminary experiments indicated that uptake was not affected by the omission of these compounds. The results of our studies indicate that the uptake of NE by synaptosomes from the hypothalamus and cerebral cortex is mediated by two saturable systems. In contrast, the uptake of 5-HT by cortical synaptsomes and DA by striatal synaptosomes, were each mediated by a single high-affinity system (see table). Our K_m values for the high-affinity transport of all 3 monoamines are lower than most corresponding values reported previously.

Substrate & Tissue	K_m (nM) 1	K_m (nM) 2	V_{max}* 1	V_{max}* 2
NE-Hypothal-P2 Fraction	85	2800	3.3	10.2
NE-Hypothal-Synaptosomes	103	2180	5.6	37.6
NE-Cerebral Cortex-Synaptosomes	73	1280	2.6	39.2
5-HT-Cerebral Cortex-P2 Fraction	31	----	5.7	----
5-HT-Cerebral Cortex-Synaptosomes	28	----	5.0	----
DA-Striatum-P2 Fraction	101	----	80.6	----
DA-Striatum-Synaptosomes	137	----	131.0	----

*pmol min^{-1} mg $protein^{-1}$: N=3 or 4

258.8 KINETIC ANALYSIS OF THE INHIBITION OF SYNAPTOSOMAL UPTAKE OF NOREPINEPHRINE, DOPAMINE, AND SEROTONIN BY McN-4612-X-11 AND SELECTED ANALOGS. C. Schneider*, R. Shank, W. Baldy*, P. Setler, J. Gardocki*, B. Maryonoff*, and D. McComsey*, McNeil Pharmaceutical, Spring House, PA

McN-4612-X-11 ((+)-1,2,3,5,6,10bß-hexahydro-6α-phenylpyrrolo[2,1-a]isoquinoline) and a variety of structural analogs reverse tetrabenazine induced depression of motor activity and ptosis in mice and rats, and inhibit the accumulation of NE, 5-HT and DA by synaptic terminals (biological effects indicative of potential antidepressant activity). As part of our evaluation of the efficacy and mechanism of action of this series of compounds, we have determined their potencies as inhibitors of synaptosomal uptake of NE, DA and 5-HT, and for a few compounds, we have undertaken a kinetic analysis to establish whether the inhibition is competitive or non-competitive. Crude synaptosomal preparations (Whittaker P2 fractions) and "purified" synaptosomes obtained from discrete regions of the rat brain were used in these studies (hypothalamus and cerebral cortex for NE, striatum for DA, and cerebral cortex for 5-HT). The results of our studies revealed that McN-4612-X-11 and other structurally similar analogs are competitive inhibitors of NE, 5-HT and DA transport. When the synaptosomal preparations were pre-incubated with the uptake inhibitors for 30 min at 37°C, the inhibition remained competitive and the potency was either unaffected or was slightly greater than in samples that were not pre-incubated. These results indicate that McN-4612-X-11 and its analogs are either not substrates for the monoamine transport carriers or are transported quite slowly. Desmethylimipramine (NE and DA uptake) and imipramine (5-HT uptake) were studied as reference compounds. They were competitive inhibitors of NE, DA, and 5-HT uptake, respectively. Surprisingly, the compounds studied were less effective inhibitors of NE uptake by "purified" synaptosomes (obtained from Percoll density gradients) than by "crude" synaptosomal preparations. This difference between the "crude" and "purified" synaptosomes did not generalize to inhibition of 5-HT and DA uptake.

258.9 MULTIPLE POOLS OF TYROSINE AND DOPAMINE IN ADRENAL CHROMAFFIN CELLS: EVIDENCE FOR PREFERENTIAL UTILIZATION OF NEWLY TAKEN-UP TYROSINE AND NEWLY SYNTHESIZED DOPAMINE IN NOREPINEPHRINE BIOSYNTHESIS. F. S. Menniti and E. J. Diliberto, Jr.*. Department of Medicinal Biochemistry, The Wellcome Research Laboratories, Research Triangle Park, NC 27709, U.S.A.

The biosynthesis of norepinephrine (NE) from the precursor tyrosine (Tyr) involves a multi-step pathway originating with cellular uptake of the amino acid from the extracellular space. The various enzymes in this pathway have been well characterized *in vitro*; however, with the exception of the vesicular localization of dopamine-β-hydroxylase, little is known about the intracellular organization of these enzymes or of the precursor pools. We have studied this organization in primary cultures of bovine adrenomedullary chromaffin cells. Cell cultures were incubated for up to 6 hr in a balanced salt solution containing 50 mM [^{14}C]Tyr in the presence or absence of drugs which inhibit catecholamine synthesis. Catecholamine and Tyr content of the cells and incubation media were analyzed by HPLC-EC and the radioactivity in each chemical species was quantified by liquid scintillation spectroscopy. After inhibition of L-aromatic amino acid decarboxylase by brocresine, the accumulated [^{14}C]DOPA had a specific activity higher than that measured for intracellular [^{14}C]Tyr but equivalent to that of extracellular [^{14}C]Tyr. This result suggests that newly taken-up Tyr is readily accessible to tyrosine hydroxylase, but equilibrates only slowly with other, as yet undefined, pools of intracellular Tyr. In the absence of drugs, there was a concomitant and equivalent accumulation of both [^{14}C]dopamine (DA) and [^{14}C]NE over time. This was accompanied by a 10-25%/hr increase in total cellular DA but only minor changes in the total cellular NE content. Catecholamines in the media amounted to approximately 5% of radioactive or total cellular content and essentially reflected the changes observed in the cells, although with considerably more variability. The pattern of change in catecholamine content is consistent with calculated rates of NE and DA synthesis based on the extracellular specific activity of [^{14}C]Tyr. In contrast, a 10-15%/hr increase in NE content and a decrease in DA content were predicted from NE synthesis rates based on the intracellular specific activity of [^{14}C]DA. This suggests that newly synthesized DA (i.e., DA of a specific activity equal to that of extracellular Tyr) is preferentially utilized as the immediate NE precursor. In cells preincubated for various times up to 4 hr with [^{14}C]Tyr, the addition of brocresine caused a rapid disappearance of cellular DA with no change in the specific activity of DA or accumulation in the extracellular media. The rate of DA disappearance from the cells after brocresine was not affected by inhibition of dopamine-β-hydroxylase. These findings are consistent with the hypothesis that after synthesis in the cytoplasm DA is rapidly taken up into the chromaffin vesicle. Upon uptake DA is either converted to NE by dopamine-β-hydroxylase coupled to the transport mechanism or is sequestered in a single pool which is not an immediate precursor for NE biosynthesis.

258.10 FACTORS AFFECTING MEASUREMENT OF ADRENALINE FORMING ENZYME IN LOCUS COERULEUS SAMPLES OF POSTMORTEM HUMAN BRAIN. W.J.Burke and H.D.Chung. St. Louis VA Med.Ctr. and St. Louis Univ.Med. Sch., St. Louis, MO 63125.

We sought to determine factors affecting measurement of phenylethanolamine N-methyltransferase(PNMT) in postmortem locus coeruleus(L.C.). Bodies were kept at 4°C within 2h of death. Postmortem delay was less than 24h. Samples were frozen immediately on dry ice and either assayed on the day of autopsy or frozen at -70°C. Each L.C. was homogenized in 10vol. of 0.15M KCl 0.2% triton x100 containing 2mM mercaptoethanol. Apparent PNMT activity was measured by a modification of a method previously described(Proc. Soc.Exp.Biol.Med.,1973,142:982). PNMT on the day of autopsy was 29.5±0.7 picomoles/h/mg tissue. In a sample frozen at -70°C for 2 days, there was no decrease in activity. There was a 42% decrease in PNMT in homogenates centrifuged at 27000xg for 30 min . Dialysis of homogenate against 1mM PO_4 buffer with 2mM mercaptoethanol did not increase activity. In the absence of mercaptoethanol, PNMT of dialyzed supernatent was reduced by 70%. PNMT measured in the presence of 100mM PO_4 was reduced by 27% compared with that measured with 4mM buffer. Optimum pH for the reaction with PO_4 buffer was 7.9 .

258.11 DOPAMINE β-HYDROXYLASE: FROM BIOSYNTHESIS TO SECRETION IN THE PC12 PHEOCHROMOCYTOMA CELL LINE. E.L. Sabban[1] and M. Goldstein.[2] [1]Dept. Biochem., New York Med. College, Valhalla, NY 10595 and [2]Neurochem. Lab., New York Univ. Med. Center, New York, NY 10016 .

Dopamine β-hydroxylase (DBH), the enzyme which catalyzes the synthesis of norepinephrine (NE), has been shown to be present in the PC12 pheochromocytoma cell line in two subunit forms. The 77K form appears to be a precursor of the 73K form (Sabban et al., 1983, J. Biol. Chem. 258, 7812). To study the regulation of this post-translational conversion, we found that it was complete, and the 73K subunit form predominated, following pretreatment of PC12 cells for several days with NGF (50 ng/ml), dexamethasone (10^{-5}M) or dibutyryl cyclic AMP (1mM) (Sabban et al., 1983, J. Biol. Chem. 258, 7819). A similar situation occurred following pretreatment of PC12 cells for 30 min with the carboxylic ionophore, monensin (100-200 μM). Monensin is known to halt the exit of secretory proteins from the Golgi apparatus in a number of systems, and is reported to deplete catecholamines in PC12 cells. The effect of monensin on DBH is proposed to effect the exit of DBH from the Golgi. The conversion of the 77K to the 73K subunit form probably occurs up to this stage in the biosynthetic pathway. Thus, this processing activity appears to be regulated.

The secretion of DBH, and other secretory proteins, from PC12 cells was studied. A portion of DBH is known to be secreted with NE from the adrenal medulla in response to various secretagogues. To study the secretion in PC12 cells, we used Ba^{+2} which has been shown to cause exocytotic secretion of catecholamines in the absence of depolarizing conditions (Greene & Rein, 1977, Brain Res. 129, 247). The PC12 cells, labeled for 6 hr with ^{35}S-methionine, were incubated with 2 mM $BaCl_2$ in phospho-buffered saline. After removal of the cells by centrifugation, the solution was concentrated in a minicon concentrator (Amicon) with a cut-off point of 15,000 daltons. The secretion of proteins (>15,000 MW) was enhanced about 6-fold by Ba^{+2}. Analysis by SDS-polyacrylamide gel electrophoresis and fluorography showed that all the proteins released at basal levels were enhanced with $BaCl_2$. DBH could be immunoprecipitated from the ^{35}S-Met proteins secreted in response to $BaCl_2$. The time course for DBH to be able to be secreted is being established.

These experiments should help in elucidating the biogenesis of chromaffin granules and neuronal vesicles. (Supported by NIH grants NS 20440 (E.S.) and NS 06801 (M.G.).

258.12 INHIBITION OF PROTEIN CARBOXYLMETHYLATION AND DOPAMINE AUTORECEPTOR FUNCTIONING. C.F. Saller* and A.I. Salama (Spon: M.E. Goldberg). Dept. of Pharmacology, Stuart Pharmaceuticals, Div. of ICI Americas, Wilmington, DE 19897.

Dopamine (DA) agonists have been reported to stimulate protein carboxymethylation (PCM) and it has been proposed that DA-stimulated PCM activity may be linked to DA autoreceptor functioning (Billingsley, M.L. and Roth, R.H., J. Pharmacol. Exp. Ther. 223 (1982) 681). We have examined the possible involvement of PCM in DA autoreceptor functioning by investigating the effects of DA agonists on DA synthesis and release in the presence and absence of PCM inhibitors. The effects of PCM inhibitors on DA synthesis regulation was studied by measuring the ability of DA agonists to inhibit the conversion of ^{3}H-tyrosine to ^{3}H-DA in rat striatal synaptosomes. In this preparation, DA, apomorphine,(APO) and other DA receptor agonists inhibited DA synthesis by a process which is antagonized by DA receptor antagonists. However, the ability of DA agonists to inhibit DA synthesis was unaffected by up to 100μM of the PCM inhibitors: S-adenosyl-L-homocysteine (SAH), L-homocysteine thiolactone and adenosine. Adding up to 100μM of the methyl donor S-adenosyl-L-methionine (SAM) also had no effect on the response to DA agonists. Furthermore, under the conditions used to measure DA synthesis, PCM activity, using ^{3}H-SAM as the methyl donor, was nearly completely inhibited by SAH; but stimulation of PCM activity by either DA, APO or 3-PPP was not detectable. Thus, DA can still regulate basal DA synthesis in the absence of readily detectable changes in PCM activity and when PCM is largely inhibited.

The effects of PCM on DA release were examined using superfused striatal slices. APO inhibited the potassium-evoked release of DA in a dose-dependent manner and this inhibition was antagonized by DA receptor antagonists. Preincubation of slices with SAH tended to reduce, but did not significantly alter the response to either 0.5 or 1.0μM APO. SAH did antagonize the response to 5.0μM APO. Interestingly, 5.0μM APO greatly suppressed both DA release and acetylcholine release, which is mediated by postsynaptic DA receptors; whereas 0.5μM and 1.0μM APO predominantly affected only DA release. Thus, the response to doses of APO which appear to be relatively selective for DA autoreceptors are not affected by SAH. These findings indicated that DA autoreceptor functioning is not drastically affected by the presence of PCM inhibitors. However, it is possible that a small pool of PCM activity exists which is not easily inhibited or measured.

258.13 INHIBITION OF PROTEIN CARBOXYL METHYLATION BLOCKS MODULATION BY DA AGONISTS OF 3H-DA RELEASE FROM BRAIN SLICES. Marina E. Wolf & Robert H. Roth, Department of Pharmacology, Yale Univ. Sch. Med., New Haven CT 06510

Protein carboxyl methylase (PCM) catalyzes the transfer of methyl groups from S-adenosyl methionine to carboxyl groups of protein substrates, producing a reversible modification of protein structure and function which has been postulated to play a role in many intracellular events, including regulation of transmitter release. We have previously demonstrated that activation of dopamine (DA) autoreceptors by DA agonists stimulates PCM activity in striatal synaptosomes (Billingsley and Roth, 1982) and slices (Wolf and Roth, 1983), suggesting a role for PCM in the transduction of DA agonist actions at the autoreceptor. The present study investigated the effects of S-adenosyl homocysteine (SAH), an inhibitor of transmethylation reactions, on the ability of DA agonists to decrease K+-stimulated release of 3H-DA from striatal slices.

Slices were loaded with 3H-DA and superfused with Krebs-Ringer-MOPS buffer (KRM). Release was stimulated by superfusing for 1 min with KRM containing 30 mM K+. Results are expressed as the ratio of the second K+-stimulated release to the first (S2/S1), with stimulated overflow of 3H-DA calculated on the basis of percent fractional release. In control experiments, S2/S1 for striatal slices was 0.99. Addition of the putative autoreceptor selective DA agonist EMD 23 448 (10 uM) to the superfusion medium 5 min before S2 reduced S2/S1 to 0.57. Apomorphine (10uM) produced a similar reduction (S2/S1=0.62). When S-adenosyl homocysteine (SAH) was included in the superfusion medium at a concentration (100uM) which produces an 80% inhibition of PCM in striatal slices, EMD and apomorphine were much less effective at decreasing K+-stimulated release (S2/S1=0.75 and 1.00, respectively). This suggests that activation of DA autoreceptors may be coupled to inhibition of DA release via a mechanism involving methylation of proteins in the presynaptic terminal. SAH alone caused only a small decrease in S2/S1 (0.87).

Previous work (Bannon et al., 1981) has demonstrated that the prefrontal cortex lacks synthesis modulating DA autoreceptors. In preliminary experiments, K+-stimulated release of 3H-DA from slices prepared from prefrontal cortex was reduced significantly by 10uM EMD (S2/S1 = 0.64) in comparison to untreated slices (0.96). This raises the interesting possibility that release and synthesis of DA may be regulated independently at distinct presynaptic sites. (Supported by USPHS Grant 14092, State of CT, and the NSF)

258.14 EFFECT OF PHARMACOLOGICAL ACTIVATION AND BLOCKADE OF Ca^{++} CHANNELS ON ENDOGENOUS DOPAMINE RELEASE FROM TIDA NEURONS. L.Annunziato,S.Amoroso‡,M.Taglialatela‡,L.Fuiano+ and G.F.DiRenzo
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Extracellular calcium ions play an essential role in the nervous system function.When cell membrane is depolarized by high K^+,Ca^{++} diffuses into the neurons through specific channels and activates neurotransmitter release. Recently it has been demonstrated the existence in the brain of specific binding sites for compounds which are able to interfere with Ca^{++} influx through cell membrane.

In this study we have investigated in vitro the effects of different classes of organic"Ca^{++} entry blockers" and of a new dihydropyridine derivative which selectively activates Ca^{++} channels on endogenous dopamine (DA)release from an arcuate periventricular nucleus-median eminence fragment containing cell bodies and nerve endings of tuberoinfundibular dopaminergic(TIDA)neurons.

Arcuate-periventricular nuclei-median eminence fragments were dissected from adult female rats under stereomicroscope and incubated in a Krebs-Ringer bicarbonate buffer and continuously aerated with 95%O_2:5%CO_2.DA released into the incubation medium was evaluated by a sensitive radioenzymatic procedure.

Verapamil and its methoxyderivative D-600,in doses ranging from 10-100 uM,blocked DA release evoked by depolarizing concentrations of K^+(35 mM).Among the dihydropyridine derivatives only nifedipine,at the high concentration of 100 uM,blocked DA-stimulated release,whereas nitrendipine and nimodipine were uneffective in doses up to 100 uM.The novel dihydropyridine analogue,methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)pyridine-5-carboxylate,which seems to be a potent Ca^{++}-channel activator,was tested.This compound at the dose of 10 uM produced a marked release of endogenous DA from TIDA neurons.

All together these results seem to suggest that Ca^{++} channels,involved in the release of DA from TIDA neurons,can be pharmacologically modulated.
(This study was supported by MPI and CNR 82.02874.04 Grants to L.A.)

258.15 WITHDRAWN

259.1 **CORRELATION OF RATES OF CALCIUM ENTRY AND ENDOGENOUS DOPAMINE RELEASE IN MOUSE STRIATAL SYNAPTOSOMES.** S.W. Leslie, J.J. Woodward*, and R.E. Wilcox. Division of Pharmacology, College of Pharmacy, The University of Texas at Austin, Austin, Tx 78712.

Recent evidence has established that voltage-dependent calcium entry into synaptosomes occurs through fast-and slow-phase processes (Gripenberg et al., Br. J. Pharmacol. 71:265, 1980; Nachshen and Blaustein, J. Gen. Physiol. 76,709, 1980; Leslie et al., J. Neurochem. 41,1602, 1983). Furthermore, Drapeau and Blaustein (J. Neurosci. 3:703, 1983) and Suszkiw and O'Leary (J. Neurochem. 41:868, 1983) have demonstrated that the temporal characteristics of potassium-stimulated calcium entry and ^{3}H-dopamine and ^{3}H-acetylcholine release agree closely. We have correlated the rates of fast- and slow-phase calcium entry with the rates of endogenous dopamine release from mouse striatal synaptosomes to examine further the relationship(s) between simultaneous calcium entry and neurotransmitter release. Voltage-dependent $^{45}Ca^{++}$ entry into and the endogenous release of dopamine from synaptosomes was initiated by the addition of 30 mM KCl. $^{45}Ca^{++}$ entry and the release of dopamine were terminated at 1,3,5,15, and 30 second time periods by the addition of an "EGTA-stopping solution". Both $^{45}Ca^{++}$ uptake and dopamine release exhibited initial fast rates through approximately 5 seconds following 30 mM KCl depolarization. Calculated rate constants for $^{45}Ca^{++}$ entry and dopamine release were 0.26 S^{-1} and 0.25 S^{-1}, respectively. The fastest rate for both processes occurred between 0 and 1 second. $^{45}Ca^{++}$ uptake and dopamine release at the 5-15 second interval dropped off to 13 and 10 percent, respectively, of the 0-1 second interval. Very little additional calcium uptake or dopamine release was observed after 15 seconds of depolarization. Calculation of the ratio of calcium entry versus dopamine release suggests that approximately 1 to 2 calcium ions are required for the release of a single molecule of dopamine. Our results suggest that calcium entry is coupled to endogenous dopamine release for both the fast- and slow-phase processes. (supported in part by NIMH grant MH33442, NIAAA grant AA05809 and by RSDA AA00044 to S.W.L.).

259.2 RAPID UPTAKE OF DOPAMINE BY MOUSE STRIATAL SYNAPTOSOMES. John J. Woodward,* R.E. Wilcox, S.W. Leslie, and W.H. Riffee. Division of Pharmacology, College of Pharmacy, University of Texas at Austin, Austin, Texas 78712.

The rapid uptake of neurotransmitters is a major mechanism for the termination of transmitter activity following release. Recently, we have reported on the coupled fast-phase influx of calcium and release of dopamine from mouse striatal synaptosomes (Leslie et al., 1984, Brain Research, in press). Under similar experimental conditions, we have examined the uptake of dopamine into mouse striatal synaptosomes over a 1-60 second time period. Uptake was linear over the one minute time period with no apparent fast component. Kinetic measurements at 15 seconds revealed a K_m of 298 nM and a Vmax of 275.6 pmoles/mg protein min^{-1}. Uptake was both temperature and sodium dependent with a half-maximal velocity at a sodium concentration of 80 mM.

Potassium induced depolarization decreased uptake at all times measured. The decrease could not be fully attributed to dilution of the tritiated dopamine by release of endogenous dopamine. The decrease, expressed as a percent of the control value, was 30% from 0-1 second, and 50-60% at all other time points. Amfonelic acid, 20 nM, decreased uptake under resting KCl conditions by 75% at all time points.

These data suggest that depolarization induced release of dopamine may decrease uptake by disrupting the membrane associated carrier uptake system within a time period that corresponds to the fast phase component of dopamine release. Supported in part by MH33442 and AA05809.

259.3 INCREASES IN DOPAMINE METABOLITE/DOPAMINE RATIOS AFTER HALOPERIDOL TREATMENT ARE LOWER IN "HALOPERIDOL-RESISTANT" GERBILS THAN IN "HALOPERIDOL-SENSITIVE" GERBILS. R. E. Wilcox[1] and M. Upchurch[2].* Departments of Pharmacology[1] and Psychology[2], University of Texas at Austin, Austin, TX 78712.

Mongolian gerbils (Meriones unguiculatus) show large inter-individual variation in their degree of responsiveness to the dopamine receptor antagonist haloperidol. Haloperidol-sensitive (HS) and haloperidol-resistant (HR) gerbils differ in exploratory behavior and nondrugged oro-facial stereotypy in a fashion consistent with the hypothesis that HR gerbils are relatively high in dopaminergic activity while HS gerbils have relatively low dopaminergic activity. We compared ratios of the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) to dopamine (DA) in the striata of HS and HR gerbils under baseline conditions and after the gerbils had been treated with 3 mg/kg i.p. haloperidol. We also examined serum haloperidol levels in HS and HR gerbils treated with 3 mg/kg i.p. haloperidol, using a radioreceptor assay.

Under baseline conditions, HR gerbils had a significantly higher DOPAC/DA ratio than did HS animals. The HVA/DA ratio was higher in HR gerbils than in HS gerbils, but the difference was not significant. After haloperidol treatment, the DOPAC/DA ratio shifted upward by only 6% in HR gerbils but by 32% in HS gerbils. The HVA/DA ratio shifted upward by 146% in HR gerbils but by 207% in HS gerbils. There was no difference between groups in serum haloperidol levels. Thus, neurochemically and behaviorally HR gerbils are more resistant to haloperidol than are HS gerbils. The results suggest that behavioral differences between HS and HR gerbils may be accounted for in part by differences in nigrostriatal dopamine function.

259.4 INFLUENCE OF ESTROGEN ON STRIATAL DOPAMINE RELEASE. Mary E. Beer* and Jill B. Becker (SPON: G.W. Dauth). Department of Psychology and Neuroscience Laboratory Bldg. The University of Michigan, Ann Arbor, MI 48109.

The influence of gonadal hormones on striatal dopamine (DA) activity has been demonstrated both behaviorally and biochemically. In this experiment, we confirm that there are estrous cycle dependent variations in amphetamine (AMPH) stimulated striatal DA release, and that ovariectomy (OVX) attenuates this response. In addition, we report that the ovarian hormone, estrogen, can influence presynaptic striatal DA activity.

Adult female Holtzman rats were maintained on a reversed light/dark cycle. Endogenous DA release from striatal tissue was studied using a continuous flow superfusion system. DA was quantified by HPLC-EC, release was normalized for the quantity of tissue in the chamber, and expressed as a per cent of baseline.

OVX ATTENUATES AMPH-STIMULATED STRIATAL DA RELEASE. The AMPH-induced increase in striatal DA release was significantly greater in striatal tissue obtained from estrous females than from OVX females ($p<0.02$). There were also estrous cycle dependent differences, with greater AMPH-stimulated striatal DA release on estrus than on diestrus 1 ($p<0.05$).

ESTROGEN POTENTIATES STRIATAL DA RELEASE. Two weeks after OVX, animals were placed in one of four treatment groups. One group received 0.1 ml oil (s.c.). The other three groups received four consecutive days of 5 µg estradiol benzoate (EB) in 0.1 ml oil (s.c.). Of these three groups, one was tested 4 hours after EB, one 24 hours after EB, and one 96 hours after EB. A significant effect of estrogen treatment on AMPH-stimulated DA release was found ($F[3,17]=3.59$, $p=0.035$). In pairwise comparisons, the group that received EB 4 hours prior to the superfusion was found to have significantly greater AMPH-stimulated DA release than all other groups ($p<0.05$). Estrogen potentiated the AMPH-stimulated DA release (relative to OVX) at 4 hours but not at 24 or 96 hours after treatment.

These biochemical results are in agreement with the behavioral results reported last year (Becker and Robinson, Soc. Neurosci. Abstr., 9, 1983) in which we found that AMPH-induced rotational behavior was attenuated by OVX and potentiated 4 hours (but not 24 hours) after estrogen treatment. We suggest that estrogen-induced potentiation of striatal DA release may underly the increased behavioral reponse to AMPH. *(Supported by NS16437 & HD05997).*

259.5 INCREASED STRIATAL DOPAMINE RELEASE ACCOMPANIES HYPOINNERVATION OF STRIATUM DURING DEVELOPMENT OR AFTER 6-HYDROXYDOPAMINE. M. Stachowiak*, J. Bruno, E. Stricker & M. Zigmond. Depts. of Biological Sciences & Psychology, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Rats sustaining lesions of the nigrostriatal dopamine (DA) system exhibit few gross behavioral impairments if 5-10% of that projection remains. DA control over striatal targets appears to be retained in such animals. Similarly, during postnatal development, DA control over striatal neurons matures well before the adult density of dopaminergic innervation is attained. These observations suggest that enhanced efficiency of dopaminergic transmission may compensate for hypoinnervation of striatum after lesions or during development. To examine this hypothesis, striatal slices (350 um) were superfused at 100 ul/min with Krebs-Ringer bicarbonate buffer, and effluent was collected in 5 min fractions and analyzed for endogenous DA by HPLC. After an initial 20 min of superfusion, slices were subjected to electrical field stimulation (2 Hz, 18 mAmp, bipolar). First, calcium-dependent efflux was measured using slices from intact, adult rats. Efflux increased to 3-5 times the pre-stimulation rate (0.85±0.15 ng/mg protein) within 10 min and then fell to approximately twice that basal rate over the next 20 min. Next, efflux was examined using slices prepared from adult animals lesioned 2-3 weeks earlier with 6-hydroxydopamine (20 ug, ivt) or from 7-10 day old rat pups. Such animals have striatal DA levels that are 10-40% of adult control values. In each case, DA efflux exhibited a gradual rise which reached adult, control values by 20-30 min. When expressed as a fraction of pre-stimulation tissue levels of DA, efflux from these slices was several times higher than the control rate by the end of the stimulation period. This increased efflux appeared to be a consequence of both increased release and decreased reuptake since the fractional DA efflux from intact adult striatal slices could not be increased by 10 uM nomifensin, an inhibitor of DA efflux, to the rate seen in hypoinnervated slices. Moreover, increased DA biosynthesis was indicated by the maintenance of DA tissue stores in the hypoinnervated slices during stimulation. These results suggest that increased DA release, decreased reuptake, and increased synthesis may serve to compensate for hypoinnervation after injury or during development.

Supported by NS 19608 and NS 18842

259.6 ENDOGENOUS DOPAMINE RELEASE FROM RAT STRIATUM IN VITRO: VARIATIONS WITH TYROSINE CONCENTRATION AND TRAIN LENGTH J.D. Milner and R.J. Wurtman, Laboratory of Neuroendocrine Regulation, MIT, Cambridge MA 02139

The release of endogenous dopamine (DA) from superfused striatal slices can be evoked by electrical pulses in a Ca++ dependent, TTX-sensitive manner. We observe that sustained DA release requires the presence of the precursor for DA synthesis, l-tyrosine. The present study examines the dose-response relationship between the amount of DA released by two trains of stimuli (60 mA, 2 ms, 20 Hz) of 600 or 1800 pulses, and the tyrosine concentration present in the superfusate.

Tyrosine (0-50 μM) dose-dependently affected the release of DA during the second stimulus (S2) of either the long or short train. In the presence of 20 μM or greater during 600 pulses, or 40 μM or greater during 1800 pulses, S2/S1 was unity indicating that these concentrations of tyrosine are the minimum required to sustain DA release under these conditions.

DA release/pulse declined with increasing train length but was independent of stimulus frequency. During 600 pulses, DA release averaged 1.00 pg/mg/pulse in the presence of 20-50 μM tyrosine, and 0.67 pg/mg/pulse during 1800 pulses in the presence of 40-50 μM tyrosine. During tyrosine-free superfusion, DA release declined at the significantly greater rate of 0.71 pg/mg/pulse (600 pulses) and 0.47 pg/mg/pulse (1800 pulses).

As the effect of tyrosine on DA release becomes manifest during S2, we propose that during an initial stimulus the contribution of stored DA to the total released per pulse can increase when the supply of newly synthesized transmitter is compromised by sub-optimal levels of extracellular tyrosine. However, this facilitation is inoperative for a subsequent stimulus unless DA synthesis has proceeded at a sufficient rate to restore the releasable pool to prestimulus conditions, which can only occur in the presence of an adequate concentration of tyrosine, which in turn is determined by the physiological activity of these neurons.

We conclude that relative contributions to DA release in striatum from synthesis and from storage can vary in accordance with tyrosine availability and neuronal activity.

259.7 IN VIVO MEASUREMENT OF RAT STRIATAL DOPAMINE RELEASE AND REUPTAKE USING THE VOLTAMMETRIC TECHNIQUE OF CHRONOAMPEROMETRY. J.O. Schenk* and B.S. Bunney (SPON: W.E. Bunney, Jr.), Yale University School of Medicine, Departments of Psychiatry and Pharmacology, New Haven, CT 06510.

In vivo voltammetric methodology has been shown to be useful for directly measuring the fluxes of the biogenic amines and their metabolites in brain tissue. However, few papers to date have used this technique to study the basic neurobiology of uptake and release. We have used the chronoamperometrically recorded concentration-time profile resulting from the release of DA by depolarizing stimuli to directly measure, in vivo, the rate of release and uptake of dopamine (DA) in the striatum of the rat. The depolarizing stimuli used to release DA consisted of local infusions of potassium (K^+) ion-containing solutions, local electrical stimulations, and electrical stimulation of the median forebrain bundle. The electrodes used to monitor the release were untreated carbon paste electrodes, stearic acid carbon paste electrodes, and uncoated and Nafion-coated 40 um diameter carbon fiber electrodes. The electrochemical parameters used were: E-app = +0.5 V vs. Ag/AgCl for 100 msec repeated every 5, 10 or 60 seconds.

DA concentration-time profiles resulting from the aforementioned stimuli were found to obey the laws of diffusion. Under control conditions the early portions of the release profile follow diffusion predictions exactly but the return to baseline is faster than that which would be predicted by diffusion considerations alone. Under conditions where uptake is markedly inhibited or blocked (by high local concentrations of K^+ or treatment with bupropion (75 mg/kg i.p.), respectively), the complete concentration time profile is predicted by diffusion suggesting that the rapid return to baseline in control animals is due to uptake processes. In both cases the rising portion of the profile is controlled by diffusion.

The method for calculating uptake rates in vivo consisted of comparing the concentration-time profile under control conditions to the concentration-time profile under conditions where uptake had been blocked. Generally, the uptake rates determined in vivo were 1 to 2 orders of magnitude faster than those reported in vitro. The functional implications of these new values will be illustrated and compared to results from typical uptake studies in vitro. Supported by Grants MH-28849, MH-25642 and the State of CT. J.S. is supported by NIMH, National Research Service Award 1 F32 MH09081-01.

259.8 METHAMPHETAMINE-INDUCED DOPAMINE RELEASE MAY BE "TOXIC" TO SEROTONERGIC AND DOPAMINERGIC NEURONS. C.J. Schmidt*, J.K. Ritter*, P.K. Sonsalla*, G.R. Hanson and J.W. Gibb. (Spon. W. Stevens) Dept. Biochem. Pharmacol. & Tox., Univ. of Utah, Salt Lake City, UT 84112

Repeated administration of high doses of the CNS stimulant, methamphetamine (METH), produce drastic, long-term depression of monoamine synthesis in the rat brain. Neostriatal tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) activity are reduced as are neostriatal concentrations of dopamine, serotonin and their acidic metabolites. Gibb et al. (N-S Arch. Pharmacol. 310:185, 1979) demonstrated that the DA synthesis inhibitor, α-methyl-ρ-tyrosine (αMT) blocks the Meth-induced depression of TH activity and that this effect of αMT is reversed by administration of L-DOPA plus the peripheral decarboxylase inhibitor, RO4-4602. These findings clearly indicate a role for catecholamine synthesis in the METH-induced depression of TH activity.

In analogous experiments, we have examined the role of catecholamine synthesis in the response of both the dopaminergic and serotonergic system to METH. Rats were administered METH (15 mg/kg) every 6 h for 5 doses alone or in combination with αMT (60 mg/kg). Other animals receiving METH plus αMT were also administered L-DOPA (50 mg/kg) and RO4-4602 (25 mg/kg). All animals were killed 18 h after the last dose. METH alone reduced neostriatal TH and TPH activity to 70 and 30% of control, respectively. When αMT was administered with METH, TPH activity was reduced to only 80% of control while TH activity remained at control levels. Coadministration of L-DOPA plus RO4-4602 with METH and αMT reversed the protective effect of αMT; neostriatal TH and TPH activity were decreased to 80 and 38% of control, respectively. The response of cerebral TPH activity to METH was also attenuated by αMT and this effect was reversed by L-DOPA plus RO4-4602. Neostriatal serotonin and 5-hydroxyindoleacetic acid concentrations changed in a pattern identical to that of TPH activity. The results suggest that catecholamine synthesis is required for the Meth-induced neurochemical changes occurring in both the dopaminergic and serotonergic systems of the rat brain after exposure to high doses of METH.

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259.9 **MESOCORTICAL DOPAMINE NEURONS: DO AGONISTS INHIBIT SYNTHESIS VIA FEEDBACK LOOPS OR RELEASE-MODULATING AUTORECEPTORS?** Matthew P. Galloway & Robert H. Roth, Pharmacology, Yale Univ. Sch. Med., New Haven CT 06510

Mesencephalic dopamine (DA) neurons projecting to the prefrontal(PF) and cingulate(CING) cortices seem to be unique in that electrophysiological and biochemical evidence suggests that these neurons have a diminished capacity for autoregulation of impulse generation and possibly DA synthesis. Since these neurons play an important role in cognition and respond to anti-psychotic drugs, it is of interest to determine the control mechanisms to which they are sensitive. Recent reports have indicated that DA agonists can inhibit DOPA accumulaiton in the PF. These studies have been interpreted to suggest that synthesis modulating DA autoreceptors exist on the terminals of these neurons. Our studies of DA release and synthesis suggest other regulatory modes and may explain the apparent dichotomy.

We have found that DOPA synthesis *in vivo* in the PF, CING, olfactory tubercles, N. accumbens, and striatum(ST) is inhibited to a similar extent (30-40%) by low doses of apomorphine (APO, 50ug/kg,sc) and by the putative DA agonists EMD-23448(3 mg/kg) and BHT-920(1 mg/kg). Although the PF and ST respond in a similar fashion, other data suggest that the regulatory properties of these terminals are distinct. Increasing the dose of APO (to 2mg/kg) has no further effect on PF or CING synthesis while the ST shows a clear dose relationship. Following the GBL-induced cessation of impulse flow (for 3 to 5 hrs), the effect of APO on synthesis was enhanced in the ST & OT (supersensitive autoreceptors) whereas in the PF, APO was now without effect. These findings suggest that DOPA synthesis in the PF is not directly regulated by the same type of interaction seen with striatal autoreceptors. GBL administered immediately prior to APO blocked the inhibitory effect in the PF and CING, whereas the effect on striatal synthesis was still evident; this suggests that impulse flow is necessary for the effect of APO and supports the role of a feedback loop or catecholamine-sensitive input to the cell body. Interestingly,other studies suggested that chloral hydrate can a) attenuate the effect of APO on PF synthesis and, in contrast to the ST, b) increase DA levels in the PF and CING, and c) does not activate synthesis in mesocortical terminals. Also,chloral hydrate blocked the disappearance of PF-DA after aMT, and APO had no effect on PF-DA decline under these conditions. In unanethetized rats, PF&CING-DA declined rapidly (T1/2=13min) and was attenuated by APO, suggesting a potential autoregulation of DA release from PF terminals.

These, and other data (Wolf&Roth,this vol.), suggest that mesocortical DA neurons are capable of autoregulating transmitter release. However, it can be hypothesized that the observed effects on synthesis are indirect and that synthesis is modulated more by factors such as impulse flow and the amount of transmitter present. The data are compatible with the notion that synthesis modulating autoreceptors are lacking on mesocortical DA neurons. Currently our data do not address the location of the release modulating mechanism but it seems to be sensitive to agents which affect impulse flow (chloral hydrate, GBL). Supported by MH14092(RHR) & MH09102(MPG).

259.10 MESOAMYGDALOID DOPAMINE NEURONS: SOME UNUSUAL PROPERTIES. C.D. Kilts*, C.M. Anderson*, D.W. Schulz and R.B. Mailman. Duke University Medical Center, Durham, NC 27710; University of North Carolina School of Medicine, Chapel Hill, NC 27514.

A detailed study of the functional significance of dopamine (DA) in the amygdala has not been made although electrophysiological, histochemical and biochemical data support a neurotransmitter role for DA in this limbic structure. In the present study, eight amygdaloid nuclei were bilaterally microdissected from coronal brain slices (300μ), and frozen at -70°C. The catecholamine content was determined using on-line trace enrichment HPLC with electrochemical detection. The presence of catecholamine-sensitive adenylate cyclases was determined in tissue homogenates using an automated HPLC procedure. An examination of the functional characteristics of the mesoamygdaloid DA system produced some surprising results. 1) There is an extremely heterogeneous distribution of DA among the amygdaloid nuclei, with the concentration in the central nucleus being 75 fold greater than that of the adjacent medial nucleus. 2) The rate of DA turnover is significantly slower in the amygdala than in the prefrontal cortex, caudate nucleus, olfactory tubercle or nucleus accumbens. 3) Presynaptic autoreceptors regulating DA synthesis, although present, have relatively low sensitivity, although the turnover rate of amygdaloid DA is increased following acute haloperidol administration (1 mg/kg, IP). 4) Unlike adenylate cyclase activity in homogenates of the caudate nucleus and nucleus accumbens, homogenates of none of the amygdaloid nuclei show stimulation of cAMP synthesis by DA or NE. This represents a major departure from the usual association of catecholamine neurons with a responsive adenylate cyclase. Moreover, the basal rate of cAMP synthesis in the central amygdaloid nucleus is higher than in any other region of brain receiving catecholamine innervation. Thus, the individual amygdaloid nuclei differ in DA content, possess long loop feedback and presynaptic autoreceptor mediated mechanisms of DA synthesis regulation, and have a relatively slow estimated rate of impulse flow. The lack of a DA responsive adenylate cyclase suggest that in the amygdala the synaptic contacts may be between DA-containing and less conventional (e.g., peptidergic) cells, and that the functional significance of synaptic transmission at these sites could be markedly different than at the synapses of other DA systems in the brain.

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259.11 TYROSINE PREFERENTIALLY INCREASES DOPAMINE SYNTHESIS IN MESOCORTICAL DOPAMINE NEURONS WITH HIGH FIRING FREQUENCY. See-Ying Tam and Robert H. Roth. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

Tyrosine availability has been suggested to influence tyrosine hydroxylation and dopamine (DA) synthesis in DA neurons under some experimental conditions. The extent to which tyrosine can exert its effect appears dependent on the firing frequency of the neurons. Recent studies have indicated that mesocortical DA neurons projecting to the prefrontal and cingulate cortices lack autoreceptors. These neurons also exhibit a faster DA turnover rate and higher basal firing rate than those DA neurons which possess DA autoreceptors (Chiodo et al., Neuroscience, 1984). Thus, it was of interest to see if tyrosine administration could differentially affect DA synthesis depending on the state of basal activity of the DA neurons.

Rats were administered tyrosine intraperitoneally one hour before sacrifice. NSD-1015, a decarboxylase inhibitor, was given 30 minutes after tyrosine administration and DOPA accumulation measured in selected brain regions. Tyrosine administration (25 mg/kg) caused significant increases in DOPA accumulation over saline controls in the prefrontal cortex (+32%) and the cingulate cortex (+29%), but not in the piriform cortex, olfactory tubercle and striatum. No significant change was observed in any DA systems at a higher dose of tyrosine (50 mg/kg). When tyrosine was administered without the decarboxylase inhibitor, a significant increase in DA level was observed in the prefrontal cortex at 50 mg/kg (+24%), but not at 25 mg/kg. No significant alterations in DA levels were observed in other brain areas. Moreover, levels of norepinephrine (NE), DOPAC and MHPG remained unchanged after tyrosine administration in all DA and NE systems examined.

This study demonstrates that a low dose of tyrosine preferentially increases tyrosine hydroxylation in the mesoprefrontal and mesocingulate DA neurons which have previously been shown to have a high basal firing frequency and to lack DA autoreceptors. Thus, dopamine neurons lacking autoreceptors (i.e., mesocortical DA neurons projecting to prefrontal and cingulate cortices) may, under normal physiological conditions, have the synthesis of their transmitter controlled in part by the availability of circulating tyrosine. (Supported in part by USPHS Grant MH-14092 and the State of Connecticut.)

259.12 BENZODIAZEPINE RECEPTOR MODULATION OF STRESS-INDUCED ACTIVATION OF PREFRONTAL DOPAMINE NEURONS. Kenan Onel*, See-Ying Tam and Robert H. Roth, Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510

There is good evidence indicating that some mesocortical dopamine (DA) neurons are activated by stress. An increase in DA metabolism is observed in these neurons after electric footshock, and such changes can be reversed by benzodiazepines. Recent studies have suggested that benzodiazepines exert their anxiolytic effect by interacting with specific benzodiazepine receptors in the brain. Thus, we examined whether these receptors could be involved in the stress-induced activation of mesocortical DA neurons.

Rats were stressed by mild electric footshock (0.2 mA, 160 ms duration, 320 ms interval) for 20 minutes. Norepinephrine, DA and DOPAC levels were measured in different brain regions by HPLC using electrochemical detection. This mild footshock paradigm caused a significant and selective increase in DOPAC level in the prefrontal cortex (+80%), but not in the other mesocortical DA projections (cingulate, entorhinal, and piriform cortices), the mesolimbic projection (olfactory tubercle), or the nigro-striatal projection (striatum). Diazepam, given at 5 mg/kg, i.p., 30 minutes before stress, completely reversed the stress-induced activation of the mesoprefrontal DA neurons. Triazolam, another anxiolytic, given at 1 mg/kg, i.p., also blocked the stress-induced increase in prefrontal DOPAC. When CGS 8216, a benzodiazepine receptor antagonist, was administered at 10 mg/kg, i.p., 15 minutes prior to diazepam treatment, the ability of diazepam to reverse the stress-induced elevation of DA metabolism in the prefrontal cortex was completely antagonized.

These studies demonstrate that stress induced by using a mild electric footshock paradigm causes a selective activation of the prefrontal mesocortical DA neurons. Furthermore, these data suggest an involvement of benzodiazepine receptors in the modulation of the prefrontal DA system. (Supported in part by USPHS Grant MH-14092 and the State of Connecticut)

259.13 *EFFECTS OF ACUTE MORPHINE ADMINISTRATION ON INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONS IN THE MALE RAT.* K.J. Lookingland and K.E. Moore. Dept. of Pharmacology/Toxicology, Michigan State Univ., East Lansing, MI 48824.

Previous studies from this laboratory have demonstrated that following acute morphine administration the rates of synthesis and turnover of DA increase in terminals of nigrostriatal and mesolimbic neurons, but decrease in terminals of the tuberoinfundibular neurons. These results suggested that morphine differentially influences the activity of the various DA neuronal systems in the brain. The purpose of the present study was to determine the effect of acute morphine administration on the activity of incertohypothalamic DA neurons located in the rostral, periventricular and caudal, dorsomedial hypothalamus.

The activities of the nigrostriatal, mesolimbic, tuberoinfundibular and incertohypothalamic DA neurons were estimated by measuring: 1) the rate of turnover of DA (decline after α-methyltyrosine, 250 mg/kg, i.p.) and 2) the concentration of dihydroxyphenylacetic acid (DOPAC) in brain regions containing cell bodies or terminals of these neurons; i.e., striatum, nucleus accumbens, median eminence and various hypothalamic nuclei, respectively. The rate of turnover of DA and the concentration of DOPAC were increased in striatum and nucleus accumbens and decreased in the median eminence 60 min after the administration of morphine (10 mg/kg, i.p.). Morphine increased the rate of turnover of DA and the concentration of DOPAC in brain regions containing both cell bodies (rostral, periventricular nucleus (A_{14}) and medial zona incerta, A_{13}) and terminals (medial preoptic, preopticosuprachiasmatic and dorsomedial nucleus) of incertohypothalamic DA neurons. These results indicate that incertohypothalamic DA neurons resemble the extrahypothalamic nigrostriatal and mesotelencephalic DA neurons in their response to morphine rather than the more anatomically-related tuberoinfundibular DA neurons. Furthermore, these results suggest that DOPAC may be used as an index of incertohypothalamic DA neuronal activity. (Supported by NIH grant NS15911.)

259.14 *DIFFERENTIAL ACTION OF BROMOCRIPTINE ON DOPAMINERGIC NEURONS IN THE STRIATUM VERSUS NUCLEUS ACCUMBENS AND OLFACTORY TUBERCLE.* A.C. Gredler*, K.E. Moore and K.T. Demarest. Dept. of Pharmacology/Toxicology, Michigan State Univ., East Lansing, MI 48824.

The present studies were undertaken to characterize the ability of the dopaminergic (DA) agonist bromocriptine (CB154) to inhibit the synthesis of DA in terminals of nigrostriatal and mesolimbic dopaminergic neurons. The in vivo synthesis of DA was estimated by the rate of accumulation of dihydroxyphenylalanine (DOPA) in the terminal regions of nigrostriatal (striatum) and mesolimbic (nucleus accumbens, olfactory tubercle) neurons 30 minutes after the administration of a decarboxylase inhibitor (3-hydroxybenzylhydrazine, NSD 1015; 100 mg/kg, i.p.). The rate of DOPA accumulation in these regions was utilized as a measure of the ability of bromocriptine to inhibit DA synthesis via DA receptor-mediated mechanisms (i.e., neuronal feedback loops, autoreceptors). The ability of bromocriptine to activate DA autoreceptors in these regions was evaluated by the ability of this drug to inhibit DA synthesis following pretreatment with γ-butyrolactone (GBL; 750 mg/kg, i.p.). Bromocriptine induced a dose-dependent (0.1-10 mg/kg, i.p.) decrease in the rate of DOPA accumulation in the striatum, nucleus accumbens, and olfactory tubercle after 1.5 h in rats pretreated with 0.9% saline or GBL. However, a time course (1.5, 3, 6 and 24 h) following a single injection of bromocriptine (10 mg/kg, i.p.) demonstrated dramatic regional differences in the ability of this drug to inhibit DA synthesis in saline versus GBL pretreated rats. Bromocriptine inhibited the GBL-induced increase in DOPA accumulation for 6 h in all regions examined. On the other hand, in the striatum of saline-treated rats the decrease in DA synthesis was evident only at the 1.5 h time interval after bromocriptine administration while in the nucleus accumbens and olfactory tubercle DA synthesis remained inhibited for 6 h. These results suggest that there is no regional difference in the ability of bromocriptine to inhibit synthesis via autoreceptor mechanisms but there appear to be differences in DA receptor-mediated neuronal feedback loops which regulate nigrostriatal and mesolimbic DA neurons. (Supported by USPHS grant and NS15911.)

259.15 SELECTIVE TOXICITY OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) ON THE A9 SUBGROUP OF DOPAMINERGIC NEURONS IN THE MONKEY AND THE DOG. R.S. Burns*, D.M. Jacobowitz, C.C. Chuieh, J.M. Phillips*, M.H. Ebert* and I.J. Kopin. Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

In primates, MPTP produces a loss of pigmented nerve cells in the substantia nigra compacta (SNC) and a parkinson-like disorder (akinesia, rigidity, tremor, a flexed posture) reversed by L-dopa (Burns, R.S., et al., Proc. Natl. Acad. Sci. USA, 80:4546, 1983).

Six rhesus monkeys were given 5 doses (0.33 mg/kg) of MPTP i.v. at 24 hr intervals and sacrificed after 1 to 5 months. Brain sections were stained for catecholamines (CA) by the glyoxylic acid method of histofluorescence or for tyrosine hydroxylase (TH) by an immunohistochemical method. Tissue samples of specific brain regions were obtained by micropunch and DA and HVA determined by HPLC.

The number of cell bodies containing CA or TH in the SNC (A9) was markedly decreased. The DA contents of the medial (36% of control) lateral (26%) and dorsolateral (8%) putamen and the medial (24%), lateral (12%) and dorsal (13%) caudate nucleus were decreased and the HVA/DA ratios increased (2-6X) with a distal/proximal gradiant. The number of ventral tegmental cells (A10) containing CA or TH appeared normal. Changes in the DA content of the nucleus accumbens (84%) and olfactory tubercule (83%) were not significant. Fluorescent nerve fibers in the median eminence appeared normal with plasma prolactin levels unchanged. A similar pattern of neurochemical changes associated with nerve cell loss in the SNC has now been found in the beagle after MPTP administration.

The selective toxicity of low doses of MPTP on the A9 cells in both the monkey and the dog suggests that a basic biochemical difference exists between the nigrostriatal and other DA systems.

259.16 DIFFERENTIAL EFFECTS OF 1-METHYL-4-PHENYL-1,2,3,6,-TETRAHYDROPYRIDINE (1-MPTP) ON RAT STRIATAL TYROSINE HYDROXYLASE (TH) AND TRYPTOPHAN HYDROXYLASE (TPH) ACTIVITIES. L.A. Matsuda*, C.J. Schmidt* and J.W. Gibb (SPON: J. Madsen). Dept. Biochem. Pharmacol. and Tox., Univ. of Utah, Salt Lake City, UT 84112.

Recently, Steranka et al. reported that 1-MPTP, a compound which consistently produces a parkinsonian syndrome in primates, causes significant decreases in dopamine and serotonin concentrations in various regions of the rat brain (Fed. Proc. 43: 586, 1984). In an attempt to further characterize the effect of 1-MPTP on the dopaminergic and serotonergic systems of the rat, we have examined the effect of two different doses on striatal TH and TPH activities and the time course of these effects. Rats received an i.p. loading dose followed by a 24-h infusion of 1-MPTP (total infusion doses of 25-mg or 50-mg) from subcutaneously implanted osmotic pumps (as described by Steranka). Rats were killed 7 days after removal of pumps and TH and TPH activities were determined by radioenzymatic assays. At 25 mg and 50 mg, TH activity was decreased to 90 and 65% of control, respectively. TPH activity was not significantly affected at either dose. In time-course experiments, rats were administered the 50 mg infusion dose and killed 1, 3 or 7 days after pump removal. TH activity was significantly depressed (84% of control) 24 h after treatment with the effect plateauing at 3 days (71% of control). TPH activity was significantly depressed only at 3 days.

In an attempt to block the effect of 1-MPTP on TH activity, rats received 2 mg/kg haloperidol (HALO) i.p. once every 6 h (4 doses) in addition to the 50 mg 1-MPTP infusion. Seven days after treatment, neither HALO nor HALO plus 1-MPTP had any effect on TPH activity. In this experiment, 1-MPTP decreased TH activity to 82% of control ($p < 0.05$). However, 1-MPTP and HALO, together, depressed TH activity to 58% of control, a significant decrease from 1-MPTP treatment alone.

These results indicate that 1-MPTP is capable of decreasing both striatal TH and TPH activities; however, the effect on TH activity is more pronounced and persists longer than the effect on TPH activity. The effect of 1-MPTP on TH activity appears to be enhanced by concurrent administration of the dopamine receptor antagonist, HALO. This observation suggests that the rate of dopamine turnover may determine the extent to which 1-MPTP depresses TH activity. This hypothesis, however, remains to be further tested. (Supported by USPHS Grants DA 00869, GM 07579 and MH 39304).

259.17 STUDIES ON THE MECHANISM OF TOLERANCE TO METHAMPHETAMINE. J.W. Gibb, C.J. Schmidt*, D.R. Gehlert*, M.A. Peat, P.K. Sonsalla*, J.K. Wamsley and G.R. Hanson. Dept. Biochem. Pharmacol. & Tox., Univ. of Utah, Salt Lake City, UT 84112.

Rats become tolerant to the neurochemical effects of high doses of methamphetamine (METH) when pretreated with gradually increasing doses of METH (Schmidt et al., Fed. Proc. 43:587, 1984). The administration of high doses of METH to naive rats results in depression of neostriatal tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) activities to 55 and 22% of control, respectively. Neostriatal concentrations of dopamine, serotonin and their metabolites are also drastically reduced. However, all changes are either blocked or significantly attenuated in pretreated animals. We now describe the results of studies examining the mechanism(s) responsible for this tolerance phenomenon.

Rats were dosed with saline or METH at 2.5, 5.0 and 7.5 mg/kg (5 doses, q6h) on days 1,3 and 5, respectively. On day 7, both groups (naive and METH-pretreated) were challenged with METH (5 x 15 mg/kg, q6h) and sacrificed 2 or 18 h later. The 18-h group was used for autoradiographic localization of [^{3}H]sulpiride binding (Gehlert and Wamsley, Eur. J. Pharmacol. 98:311, 1984) and measurement of substance P (SP) - like immunoreactivity in the substantia nigra. Forebrains from animals killed at 2 h were used for the determination of brain levels of METH, amphetamine and p-hydroxy metabolites of the two drugs by capillary gas chromatography-mass spectrometry (Peat, in preparation).

As reported previously (Ritter et al., JPET, in press) the challenge dose of METH elevates nigral SP concentrations by approximately 50%. Although the challenge dose elevated nigral SP 33% in pretreated rats, the change was significantly less than that observed in naive animals. METH reduced [^{3}H]sulpiride binding, believed to represent binding to D_2 dopamine receptors, in the neostriatum and nucleus accumbens of naive rats by 30 and 37%, respectively. The binding in pretreated animals was not significantly different from control in either brain region. Forebrain concentrations of METH and its N-demethylated metabolite, amphetamine, were reduced by 50 and 36%, respectively, in pretreated compared to naive rats after the METH challenge. The results demonstrate that tolerance to METH is manifested by a reduction in dopaminergic transmission in several brain regions and may be due to changes in the disposition or metabolism of METH after prolonged administration. (Supported by USPHS Grants DA 00869, GM 07579and MH 39304 and MH 37762).

259.18 EFFECTS OF AMPHETAMINE ON TETRAHYDROBIOPTERIN IN CAUDATE: BILATERAL ASYMMETRY STUDY. E.H.Y. Lee* and A.J. Mandell Inst. of Biomed. Sci. Academia Sinica. Taipei, Taiwan, R.O.C. and Dept. of Psychiatry, Univ. of California at San Diego. La Jolla, CA 92093 U.S.A.

Tetrahydrobiopterin (BH_4) functions as a cofactor for tyrosine hydroxylase (TOH) and tryptophan hydroxylase (TPOH) (Kaufman 1959, 1963) in regulation of monoamine biosynthesis in neural and adrenomedullary tissues. Under normal situations, brain BH_4 concentration has been shown to vary around 3 uM (Guroff et al., 1967; Lloyd and Weiner, 1971) and has a Km orders of magnitude below that of TOH and TPOH for BH_4 (Oka et al., 1982; Okuno and Fujisawa, 1982; Nakata and Fujisawa, 1982). Previous reports have also shown that among 30 psychoactive drugs studied, only amphetamine produced a significant and replicable effect on caudate BH_4: a dose-dependent decrease with a maximum decrement of about 35% (Mandell et al., 1980). To further understand the pharmacological mechanism of amphetamine on BH_4, bilateral asymmetry of caudate BH_4 were examined following systemic amphetamine administration. Caudate dopamine (DA), serotonin (5-HT) and their Precursors tyrosine and tryptophan were also studied.

Thirty-two male Sprague-Dawley rats (200-250g) were used. Twelve of them were used for BH_4 study and were randomly divided into two groups of six rats each. The other twenty rats were also divided into two groups of ten rats each for monoamine assays. The control groups received 1 ml/kg saline injection and the drug groups received 1 mg/kg amphetamine sulfate injection (s.c.) 1 hr before sacrifice. The caudate nuclei were collected for radioenzymatic assay of BH_4 or HPLC fluorescence determination of DA, 5-HT and their precursors. The results showed that there is a bilateral asymmetry of caudate BH_4 in normal rats (approximately 21%) and amphetamine decreased this asymmetry significantly (about 8%). There is about 10% absolute difference between left-right DA and 5-HT levels in caudate and amphetamine had no obvious effects on this asymmetry. Tyrosine and tryptophan showed little left-right difference (about 4%); however, amphetamine had a tendency to augment this asymmetry (about 9%). These results confirm and further suggest the uniqueness of the effects of amphetamine on brain reduced cofactor activity. As asymmetry is concerned, these results also indicate a dissociation between brain BH_4 activity and the monoamines and precursors it regulates under the influence of amphetamine. The unique effects of amphetamine on caudate BH_4 and the bilateral asymmetry may have its functional significance and clinical implications. Supported partly by a grant from the W.M.Keck Found.

259.19 NEUROCHEMICAL BASIS FOR THE USE OF 6F-DOPA FOR VISUALIZING DOPAMINE NEURONS IN THE BRAIN BY THE POSITRON EMISSION TOMOGRAPHY. C. C. Chiueh, K. L. Kirk*, M. A. Channing* and R. M. Kessler*. NIMH/NINCDS, NIADDK and NIH Nuclear Medicine Dept., NIH, Bethesda, MD 20205.

Our previous study indicates that 6-fluorocatecholamine, by fulfilling the criteria for adrenergic false transmitter, may be useful in positron emission tomographic scanning of the adrenergic nervous system in the brain or in the peripheral sympathetic nerves (Chiueh et al., J. Pharmacol. Exp. Ther., 225: 529, 1983). In the present study, we synthesized tritium-labeled 6F-dihydroxyphenylalanine (Kirk and Creveling, Medicinal Res. Rev., 4:189, 1984; 7,8-^{3}H-6F-dopa, 2.6 c/mmole) for investigating the metabolism of this compound in the plasma and the caudate nucleus.

Rats (200 g) were pretreated with a peripheral decarboxylase inhibitor (MK-486, 75 mg/kg, i.p., 60 min) and sacrificed at various times after the administration of ^{3}H-6F-dopa (300 uc per rat, i.v.). In the plasma, ^{3}H-6F-dopa levels fell rapidly and bi-exponentially with half-lives (T1/2) of 5 and 20 min, respectively. ^{3}H-3-methoxy-6F-dopa peaked at 20 min (about 180,000 cpm/ml) and decreased with a T1/2 of approximately 80 min. In the caudate nucleus, tritium counts associated with 6F-dopa, 6F-dopamine, 6F-dihydroxyphenylacetic acid (6F-dopac), 3-methoxy-6F-dopa and 6F-homovanillic acid were detectable. The major peaks were 6F-dopamine (295 cpm/mg, peaked 30 min) and 6F-dopac (97 cpm/mg, peaked 15 min). Striatal levels of other metabolites of ^{3}H-6F-dopa were less than 100 cpm/mg throughout the experimental period. There was no sign of accumulation of ^{3}H-3-methoxy-6F-dopa in the brain. The total counts consisted mainly of 6F-dopamine (about 60%). It reached 500 cpm/mg 30 min after ^{3}H-6F-dopa and decreased with a T1/2 of 70 min. The estimated rate of accumulation of 6F-dopamine in the caudate nucleus was about 75 fmole/mg/15 min while its rate of disappearance was approximately 25 fmole/mg/15 min.

Since there is no significant accumulation of 3-methoxy-6F-dopa, it is reasionable to postulate that most of the radioactivity in the human brain detected by the positron emission tomographic scanner following the administration of ^{18}Fluorine labeled 6F-dopa (Garnett et al., Nature, 305:137, 1983) was contributed mainly by 6F-dopamine. The present result also indicates that ^{18}Fluorine-labeled 6F-dopa is a better tracer than ^{11}Carbon-labeled dopa for imaging dopamine and/or its turnover rate in the brain.

260.1 ELECTRON MICROSCOPIC LOCALIZATION OF NEURON-SPECIFIC ENOLASE (NSE) IN NORMAL AND NEOPLASTIC CELLS IN THE BRAIN. S. A. Vinores*, L. J. Rubinstein*, and P. J. Marangos (SPON: S. R. VandenBerg). Div. of Neuropathol., Dept. Pathol., Univ. Virginia School of Medicine, Charlottesville, VA 22908.

The cerebrum, cerebellum, and brainstem of the rat and mouse were examined immunocytochemically for the glycolytic isoenzyme NSE. In the cerebrum, neuronal perikarya and dendrites were stained. Staining was absent in presynaptic terminals of axo-dendritic synapses. In the cerebellum, staining was seen in the perikarya, axons and parallel fibers of the granular neurons, in Golgi cell dendrites, in perikarya and axons of basket cells, and in mossy fibers. Purkinje cell perikarya and their dendrites, and climbing fibers were negative. Brainstem neurons were positive. Most myelinated axons in the cerebrum and cerebellum were negative. Fibrillary astrocytic processes between myelinated axons in white matter were positive, but all other non-neuronal cells were negative. In the positively staining cells and their processes, staining was dispersed through the cytoplasm and absent from nuclei, the interior of mitochondria, the cisternae of the Golgi complex, myelin lamellae and most membranes. Microtubules in some myelinated axons appeared decorated with reaction product.

NSE was also demonstrable by electron immunocytochemistry in a number of primary and metastatic brain tumors composed of cells whose normal counterparts do not contain NSE by light microscopy. The tumors included glioblastomas, astrocytomas, choroid plexus papillomas, and metastatic carcinomas. The intracellular distribution of NSE in neoplastic cells differed from that found in normal cells (neurons and cells of the APUD system). In addition to cytoplasmic staining, NSE was found on cell surface membranes and on membranes of organelles in both primary CNS and metastatic tumor cells, even where cytoplasmic staining was not evident. In astrocytomas and glioblastomas, cytoplasmic filaments were also decorated. The cell population of a particular tumor did not stain uniformly. Unstained cells were often found among stained cells, and the localization of the reaction product (cytoplasm, membranes or intracellular filaments) varied among cells from the same tumor. This study shows that neoplastic transformation not only seems to be accompanied by the appearance of NSE, but that the enzyme appears at intracellular sites in which NSE is not found in the normal cell.

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260.2 DECREASED PYRUVATE DEHYDROGENASE COMPLEX ACTIVITY IN HISTOLOGICALLY NORMAL ALZHEIMER BRAIN. R.K.F. Sheu*, Y.T. Kim*, J.P. Blass and M. Weksler*. Cornell Medical College and Burke Rehabilitation Center, White Plains, NY 10605.

Previous reports indicating reduced activity of the pyruvate dehydrogenase complex (PDHC) in histologically abnormal Alzheimer frontal cortex prompted us to examine PDHC in a histologically normal area, occipital cortex (in samples obtained from Dr. Peter Davies, Albert Einstein Medical College). PDHC was as reduced compared to controls in occipital cortex (10.7 $\pm$ 1.8 vs 2.9 $\pm$ 1.2 nmol/min/mg protein, mean $\pm$ SEM, $P<0.01$) as in frontal cortex (10.6 $\pm$ 0.8 vs 3.8 $\pm$ 1.2, $P<0.001$). Another mitochondrial enzyme, glutamate dehydrogenase, had the same activity in Alzheimer as in control brain.

With polyclonal antibodies, raised against beef kidney PDHC, Western blots revealed no apparent difference in sizes between Alzheimer and control enzymes. Enzyme activity titration studies indicated the presence of reduced amounts of an antigenically normal PDHC. Results of enzyme-linked immunoabsorbent assay were consistent with that conclusion. Patients and controls were well matched for age and time until autopsy. Correlations of enzyme activity with time until autopsy were poor, and the exogeneously added PDHC did not lose appreciable activity when incubated with control and Alzheimer brain homogenates.

These observations indicate that the reduction in PDHC activity in Alzheimer brain is not simply a result of anatomic damage. The reductions are more profound than reported for other energy metabolic enzymes and of the order reported for choline acetyltransferase and somatostatin. Other studies suggest the decreases in pyruvate oxidation may relate to loss of neuronal calcium homeostasis (Gibson & Peterson, 1983; Baudry et al, 1983) as well as to the cholinergic lesion.

260.3 ACUTE AMMONIA INTOXICATION ALTERS ENERGY METABOLISM IN THE RETICULAR FORMATION IN TUPAIA GLIS. D.W. McCandless*, and G. Looney (SPON: J. DeFrance). Dept. Neurobiol., Univ. of Texas Medical School at Houston, Houston, Texas 77025.

Ammonia is elevated in patients with hepatic encephalopathy, and is thought to be an important toxin in the production of neurological symptoms. Previous in vivo studies have shown the brainstem in NH_3-induced comatose animals have decreased ATP and phosphocreatine levels. Recent studies demonstrate that this effect on brainstem energy metabolism is localized to the reticular formation, and significant changes occur during the precoma phase. The present study was intended to extend these observations on NH_3-induced coma to the lower primate, the tree shrew (Tupaia glis). Accordingly, female tree shrews weighing 130-170 grams were injected ip with ammonium acetate at a dose of 20 µmoles per gram. Controls received either saline or no injection. Animals were sacrificed in a 915 megahertz microwave oven at 23 Kw 3 minutes following injection (precoma) or 1 minute following the onset of coma (9.5 minutes, range 4-12 minutes). The brains were removed and stored until further use at -80°C. Glucose, glycogen, lactate, ATP, and phosphocreatine were all measured in 100-500 nanogram samples freehand dissected from freeze-dried sections of the midbrain reticular formation. Metabolite analysis included enzymatic cycling to enhance fluorescence. Results showed a significant decrease in glucose and glycogen during the coma stage; glycogen was also decreased in the precoma phase. Lactate was increased in the coma period only, whereas phosphocreatine was decreased in both the precoma and coma stages. ATP was not significantly decreased in either precoma or coma. The sum of ATP and phosphocreatine was decreased by 50% in precoma and comatose animals. These results showing an effect of acute NH_3-intoxication on energetics in cells of the reticular formation are in general agreement with previous studies on mice with NH_3-induced encephalopathy, both showing an effect on energy metabolism in the reticular formation. Since the sum of ATP and phosphocreatine in the present study is reduced by 50%, these data are consistent with the concept that an energy decrease in reticular formation cells acts to place the animal in coma. Further, these changes occur in the precoma phase while the animal is drowsy. Onset of coma may act in a beneficial way by placing the organism in a mileau which is conducive to the correction of its threatened energy reserves. Supported in part by USPHS grant N.S. 17130.

260.4 EXPRESSION OF CLASS I MHC ANTIGENS BY PRIMARY MURINE NEURONAL CULTURES. P.T. Manning, E.M. Johnson, Jr., M.A. Palmatier,* C.L. Wilcox,* and J.H. Russell.* Dept. of Pharmacology, Washington Univ. Med. School, St. Louis, MO 63130.

Experiments were conducted to determine whether neurons in culture express class I antigens of the major histocompatibility complex (MHC) and whether neurons can serve as targets for immunological attack mediated by cytotoxic T lymphocytes (CTL) which recognize class I antigens. Primary cultures of C3H/He ($H\text{-}2^k$) or C57BL/6 ($H\text{-}2^b$) sympathetic neurons (SCGN) were obtained from the superior cervical ganglia (SCG) of neonatal mice and were grown in the presence of varying concentrations of nerve growth factor (NGF) for 2-3 weeks. These neurons were then used as targets for cloned populations of CTL generated from C57BL/6 anti-DBA/2 mixed lymphocyte cultures. Killing was monitored morphologically since both ^{51}Cr and ^{111}In were either not retained and/or were toxic to the SCGN cultures. Using high effector concentrations (CTL-3) near complete destruction of C3H neurons expressing the class I antigens that the CTL recognized occurred with 2 hrs, while C57BL/6 neurons (syngeneic to the CTL) were not lyzed. Neurites first appeared grainy and then underwent degeneration. Concentrations of NGF as high as 5 µg/ml did not block lysis. At the ultrastructural level, nuclear changes characterized by striking alterations in the distribution of chromatin (Russell et al., 1982) and apparent "blebbing" of the cytosol occurred in CTL damage neurons. These experiments demonstrate that neurons in culture do express class I antigens and can thus serve as targets for CTL and that NGF does not block the expression of class I antigens of the MHC on neurons grown in culture.

260.5 BINDING OF ANTI-MYELIN IgM M-PROTEINS TO PERIPHERAL NERVE GANGLIOSIDES. L. Freddo*[a], M. Saito*±, L. Marcala*±, R. Yu±, and N. Latov*[a] (SPON: M. Lehman[a]). Depts. of Neurology, Columbia University College of Physicians and Surgeons, New York, N.Y. 10032[a] and Yale University School of Medicine, New Haven, CT 06510±.

In some patients with neuropathy and plasma cell dyscrasia there are IgM M-proteins that react with peripheral nerve myelin and that may cause the neuropathy. These M-proteins are thought to bind to a carbohydrate moiety that is shared by the myelin associated glycoprotein (MAG), a number of other peripheral nerve myelin glycoproteins, and gangliosides, but it is not known to which of these the M-proteins bind in situ.

To study the binding of these M-proteins to gangliosides, human peripheral nerve was obtained at autopsy and myelin separated by sucrose density centrifugation. Gangliosides were prepared from whole nerve or myelin by chloroform-methanol extraction and purification by chromatography on DEAE-sephadex and Iatrobeads. Binding of M-proteins to gangliosides was examined by immunostaining after separation of gangliosides by thin layer chromatography, coating with 0.1% polyisobutylmethacrylate, and incubating with patient serum followed by peroxidase-conjugated antibodies to human IgM. In one case the serum was counterstained with a mouse monoclonal antibody to the M-protein idiotype. Immunoreactants were visualized with diaminobenzidine.

The IgM from five patients with anti-myelin M-proteins bound to a single ganglioside band present in extracts of whole peripheral nerve but not in the myelin extract. This ganglioside migrated on thin layer plates in the region between GM1 and GD1a using chloroform: methanol:0.1%CaCl2 (55:45:10) as the developing solvent. The same binding was observed when a patient's serum was counterstained with the anti-idiotype antibody to that patient's M-protein, indicating that it was the M-protein rather than other IgM species present which bound to the ganglioside band. Control sera from normal subjects and from patients with neuropathy and IgM M-proteins that did not bind to myelin did not react with the same ganglioside band.

The data indicate that anti-myelin M-proteins from patients with neuropathy and plasma cell dyscrasia, also bind to a ganglioside present in a peripheral nerve fraction other than myelin. The identity and location of this ganglioside and its role in the neuropathy is currently under investigation.

260.6 CEREBRAL ISCHEMIA IN RABBIT: IMMUNOHISTOCHEMICAL INVESTIGATION. K. Yamamoto*, T. Yoshimine* and T. Yanagihara. Dept. of Neurology, Mayo Clinic, Rochester, MN 55905.

A new experimental model of cerebral ischemia was developed in rabbits by transorbital occlusion of intracranial arteries. Under general anesthesia with ketamine hydrochloride and controlled respiration, a middle cerebral artery (procedure 1), a middle cerebral and bilateral anterior cerebral arteries (procedure 2), or a middle cerebral, ipsilateral internal carotid and bilateral anterior cerebral arteries (procedure 3) were occluded with metal clips. Postoperatively, each rabbit was observed from 30 min to 12 hrs for evolution of cerebral infarction. Four rabbits were taken for each time interval. Each brain was fixed in alcohol-5% acetic acid and embedded in paraffin. For visualization of neuronal cell bodies, dendrites and neuropils, an immunohistochemical method for tubulin was used, and for evaluation of intracerebral circulation, transcardiac perfusion of India ink was carried out.

Thirty minutes after the procedure 1, arterial perfusion on the occluded side was still normal. However, markedly reduced perfusion was seen in a wide area of the cerebral cortex and basal ganglia of the occluded side 30 min after the procedure 3. With the procedure 1, evidence of cerebral ischemia or infarction could not be observed immunohistochemically until 6 hrs after occlusion, at which time all 4 rabbits had lesions in the basal ganglia manifested as loss of the immunological reaction in the neuronal cell bodies and neuropils, while 3 out of 4 rabbits showed lesions in the parietal region of the cerebral cortex manifested as loss of the immunological reaction in the dendrites, neuropils and neuronal cell bodies. After 8 hrs, cortical lesions were observed in all 4 rabbits. With the procedure 2, all 4 rabbits developed infarction in the basal ganglia 3 hrs after occlusion but only 2 developed cortical infarction at that time. It took 6 hrs before all 4 rabbits developed cortical infarction. With the procedure 3, all 4 rabbits developed infarction in the basal ganglia and cerebral cortex one hr after occlusion. Evolution of cerebral infarction was significantly slower when the staining with hematoxylin-eosin was used for evaluation.

The present experimental model provides regional cerebral ischemia of variable degree with rabbits, which is useful for morphological, physiological and biochemical investigation of cerebral ischemia. (Supported by the grant NS-06663 from NIH).

260.7 CISPLATINUM: NUCLEAR AND CYTOPLASMIC TOXICITY IN HUMAN GLIOMA DERIVED TUMOR CELLS. M.A. Greenwood-Oberc*, B.H. Smith+, C. Pepin*, J.A. Ellis, C. Gibson, & P.L. Kornblith, Surgical Neurology Branch, NINCDS, & Biomedical Engineering & Instrumentation Branch, DRS, NIH, Bethesda, MD, 20205; + and Memorial Sloan Kettering Cancer Center & Dreyfus Medical Foundation, NY, NY 10021.

Cisplatinum (cis-dichlorodiammine platinum II)) is an established solid-tumor chemotherapeutic agent. It is thought to induce cross-link formation in DNA, thereby preventing DNA replication in tumor cells. It has an advantage as an anti-glial tumor agent over a nitrosourea such as BCNU in that it is not subject to tumor cell alkylation repair processes. To clarify its mode of cytotoxicity and to compare its actions to those of AZQ (an alkylating agent with mitochondrial toxicity) and BCNU (an alkylating agent with peroxidase suppression properties) we examined the ultrastructural changes in three glioma-derived cell-lines shown by microcytotoxicity assay to be responsive to the drug. Cells were exposed to 25,50 and 100 μg/ml of cisplatinum dissolved in F-10 medium for 4,8,15,24,36,48, & 72 hour intervals. A minimum of 50 cells for each time interval & concentration were quantitated manually and by a Bausch & Lomb FAS II image processing system. Four findings were evident. After 4 hours, affected cells became rounded with an altered nuclear-cytoplasmic ratio secondary to induced cytoplasmic loss (released as vesicles). Nuclear chromatin clumping, dilation of smooth endoplasmic reticulum and golgi swelling & vesiculation occurred after prolonged exposure. In contrast to our findings for AZQ in previous studies, no mitochondrial changes were seen at any time interval studied. With 100 μg/ml cisplatinum at 15 hrs. punctuate chromatin clumping along the periphery of the inner leaflet of the nuclear membrane occurred in at least 65% of the cells in all 3 cell lines examined. Swelling & vesiculation of perinuclear golgi were found in 75% of the cells & more than 86% of the cells contained greatly dilated smooth endoplasmic reticulum localized primarily at the periphery of the cells near, but not in contact with the plasma membrane. After 72 hrs. of exposure to 25 μg/ml cisplatinum, 76% of the cells in all 3 lines had nuclear chromatin changes, 76% contained abnormally appearing golgi and more than 78% had dilated endoplasmic reticulum. While DNA cross-linking may be important to the mechanism of cisplatinum's malignant glial cell cytotoxicity, cytoplasmic and membrane events (unique to cisplatinum as compared to BCNU and AZQ) occurring concurrently with the nuclear changes are also relevant.

260.8 LYSOSOMAL ENZYME INHIBITORS CAUSE THE ACCUMULATION OF CEROID-LIPOFUSCIN AND DOLICHOLS IN RAT BRAIN. G. Ivy, L.S. Wolfe, K. Huston*, M.Baudry and G. Lynch. Dept. of Psychobiol, Irvine, CA 92717 and Montreal Neurol. Inst., Quebec, Can. H3A 2B4

We previously reported that administration of the lysosomal thiol protease inhibitor leupeptin to rats induces the formation of ceroid-lipofuscin (CL) in hippocampus (Neurosci. Abs.9:926,'83). Since CL accumulates in aging and several diseases, notably the inherited Neuronal Ceroid Lipofuscinoses (NCL), we have suggested that our findings may provide insight into the processes responsible for these conditions. Here we examine 1)the substances induced by leupeptin, the serine protease inhibitor aprotinin and the lysosomal enzyme inhibitor chloroquine with light and electron microspy and 2)the brain dolichol content, a biochemical index of NCL and Alzheimer's disease (Kin et al,J. Neurochem 40: 1465, '83) from rats treated with inhibitors as compared to control rats. Rats received continuous infusion of saline or one of the inhibitors via osmotic pumps attached to cannulae implanted in the lateral ventricle. Two weeks later the rats were prepared for light or electron microscopy or for biochemistry. The leupeptin-induced CL has many staining properties in common with that found in normal aged rats and displays a yellowish autofluorescence. It is widely distributed in the brain, notably in hippocampus, cerebellum and neocortex. The cerebellum also displays a marked Purkinje cell loss. The chloroquine induced CL has similar staining properties and is autofluorescent in hippocampus; further analysis is in progress, as is analysis of the aprotinin treated rats. The fine morphology of the leupeptin-induced CL is similar to that seen in the NCL diseases, consisting of a granular matrix associated with curvilinear bodies and lysosomes. The chloroquine-induced CL is heterogeneous but exhibits a high proportion of membranous whorls similar to those seen in Tay Sachs disease. The biochemical analysis showed that most brain regions in rats recieving leupeptin or chloroquine, but not aprotinin had significantly increased levels of dolichols over that found in control rats. In summary, the inhibition of thiol proteases or of lysosomal enzymes causes the accumulation in neurons of material similar to that seen in two lysosomal storage diseases and ageing. In particular we have found a similarity in the brains of leupeptin treated rats and patients with NCL disease with regard to the fine morphology of the CL, the loss of Purkinje cells and the abnormal accumulation of dolichols. Our findings suggest that the enzyme detect(s) in the NCL diseases may lie in a lysosomal protease. Supported by NIA:AG-00-538-08 and MRC:MT-1345.

260.9 *GLUCOSE METABOLISM AND PLASMA MEMBRANE STUDIES IN MYOTONIC DYSTROPHY.* Hudson,A.J., Miller,M.E.* and Wright,C.G.* Dept. of Clinical Neurological Sciences, University of Western Ontario, London, Ont. N6A 5A5, Canada.

Myotonic dystrophy (MyD) is a generalized metabolic disease with the characteristic clinical features of myopathy, cataract, testicular atrophy, insulin resistant diabetes mellitus and increased catabolism of immunoglobulins. Insulin binding to MyD monocytes and fibroblasts under optimal binding conditions (16°C, pH 7.6-8.0) have reduced receptor affinity. Compatible with this is decreased insulin-stimulated transport of the non-metabolizable sugar 2-deoxyglucose (37°C, pH 7.4) into freshly biopsied MyD adipose tissue (Mably et al,J.Neurol.Sci.52:11,1981). With cultured fibroblasts under standard incubation but glucose deprived conditions (37°C, pH 7.4, 5% CO_2 in air) both basal and insulin stimulated glucose uptake in MyD cells are increased (9.97± 0.984 and 17.09±1.70 nmoles glucose/mg protein/20 min, respectively) compared with control cells (8.10±0.65 and 12.69± 1.07 nmoles glucose/mg protein/20 min; $p<0.01$). When the atmospheric CO_2 in the incubation environment of the cultured fibroblasts is raised to 20% in air or the pH is lowered to 6.5 using pH adjusted buffers, both basal and insulin-stimulated glucose uptake are then reduced similar to the decrease in adipose tissue. The most striking effect is observed with insulin-stimulated glucose uptake as indicated by the insulin-stimulated (IS) to basal (B) ratio. At pH 7.4 the IS/B ratio for MyD cells is 1.71:1 which is significantly greater than the ratio of 1.57:1 for control cells ($p<0.01$). With lowering of the pH the IS/B ratio decreases but to a greater degree in MyD cells. At pH 6.5 there is no insulin-stimulation in MyD although significant stimulation (IS/B 1.15:1;$p<0.05$) is still present in control cells. These findings suggest that physicochemical conditions of the cellular milieu can markedly affect glucose uptake in MyD due, possibly, to alterations in the insulin receptors and/or plasma membrane. We examined MyD cultured fibroblast plasma membranes by ^{125}I and ^{3}H-leucine protein labeling and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). No consistent differences from normal were found. ^{125}I-insulin was then cross-linked to cultured fibroblast plasma membrane insulin receptors and the 130 and 92 KD (α&β) subunits of the insulin receptor were identified by SDS-PAGE and appeared identical in MyD and control cells. It is concluded that insulin-stimulated glucose uptake is abnormal but SDS-PAGE shows no abnormality of insulin receptors or the plasma membrane proteins in MyD fibroblasts.

260.10 WITHDRAWN

260.11 THE FATE OF REACTIVE AXONAL SWELLINGS OCCURRING WITH HEAD INJURY. J.T. Povlishock and D.P. Becker, Dept. of Anatomy and Div. of Neurological Surgery, Med. Coll. of Va., Virginia Commonwealth Univ., Richmond, VA 23298.

Reactive axonal change is a finding common to head-injured animals and humans. Recently, we rejected the classical neuropathological belief that such reactive axonal change was the result of the traumatically-induced tearing of axons with the subsequent extrusion of their axoplasm. Rather we demonstrated that trauma induced a focal impairment of axoplasmic transport which over a 24h course resulted in an accumulation of organelles with axonal expansion to form a large reactive swelling (Povlishock et al., J. Neuropath. Exp. Neurol., 42: 225-242). The purpose of the present investigation was to evaluate the fate of such reactive swellings. To explore this issue reactive axonal change was studied over a 21 day period in cats subjected to a fluid-percussion brain injury. To aid in the recognition of the reactive axonal change, the anterograde axonal transport of horseradish peroxidase (HRP) was utilized based upon our previous experience that such anterograde HRP passage was a sensitive probe for the detection of traumatically induced axonal change. At the designated survival time,the animals were perfused with aldehydes, reacted for the visualization of the HRP,and prepared for LM and TEM analyses. Within 24h of injury,HRP-laden swellings could be recognized throughout the brainstem. Typically such swellings contained numerous mitochondria and HRP-containing tubular and vesicular profiles. Some of the swellings were encompassed by a thinned myelin sheath, while others lost their myelin investment. At 72h, the swellings showed no further increase in size; however, other forms of change were seen. Some reactive swellings demonstrated complex lobulation with neurofilamentous hyperplasia or increased electron density. Such changes were consistent with a degenerative response,and with increased survival, macrophages could be identified directly applied to the swollen axolemma. Other reactive swellings revealed little change over the 21 day course, while other swellings demonstrated elaborate regenerative sprouts. Both the myelin-invested and non-invested swellings demonstrated sprouts which arose directly from the swelling or from a point more rostral along the axonal shaft. By the 21st day,such regenerative sprouts appeared to have elongated and followed a directed course through the brain parenchyma. The results of these studies indicate that some traumatically-induced reactive swellings may undergo degeneration, while others initiate a remarkable regenerative response. Supported by NIH NS-20193.

260.12 INITIAL SEGMENT STAINING IN NEURONAL STORAGE DISEASE ESTABLISHES THAT MEGANEURITES ARE OF AXON HILLOCK ORIGIN AND DISTINCT FROM AXONAL SPHEROIDS. S.U. Walkley and A.L. Pierok* Dept. of Neuroscience, Rose F. Kennedy Center, Albert Einstein College of Medicine, Bronx, N.Y. 10461

In the original description of ganglioside storage disease (GSD) a century ago, Bernard Sachs observed enlargements on cortical neurons which he believed were abnormalities in the basilar dendritic system. More recent work using the Golgi method has suggested that cortical pyramidal neurons in human and feline GSD develop these enlargements (meganeurites) and/or neuritic sprouts (secondary neurites) at the axon hillock region. With EM these structures have been shown to be postsynaptic to afferents of unknown origin. Other EM studies have suggested that meganeurites are proximal to axonal initial segments (IS) but sampling difficulties have precluded systematic evaluation.

We have sought to systematically explore the relationship of meganeurites, axonal IS's and axonal pathology (spheroid formation) by using the ferric ion-ferrocyanide IS stain of Waxman and Quick (Brain Res. 144:1-10). Small blocks of cortical tissue from cats with GM1 GSD were fixed for EM, stained and embedded in plastic. Thick sections were counterstained with safranin O to demonstrate ganglioside-rich inclusions in somata and meganeurites without obscuring the IS stain. Results clearly indicated that the vast majority of meganeurites, identified by safranin positive staining and location adjacent to nucleus containing cell bodies, were proximal to IS's. Axonal spheroids, in contrast, appeared granular and safranin negative and occurred distal to IS staining. EM evaluation of thin sections further characterized these differences.

Our findings confirm the view that meganeurites (and secondary neurites) are of axon hillock origin. As such, meganeurites are not appropriately equated with spheroids or axonal "torpedoes" as some studies have implied. We also suggest that this same situation may exist in other storage diseases displaying similar Golgi morphology. The precise location of meganeurites and secondary neurites and, consequently, of their associated synaptic input, is critical information in the pursuit of the functional implications of such geometrical and connectivity alterations.

We thank Dr. H.J. Baker for the GM1 cat tissues and the NIH (NS-18804) and the N.Y. State Chapter of the National Tay-Sachs and Allied Diseases Association, Inc., for support.

260.13 NEUROFILAMENTS (NF) AND VIMENTIN FILAMENTS (VF) OF CULTURED NEURONS: IMMUNOCHEMICAL STUDIES OF RAT SYMPATHETIC NEURONS (RSN) AND A CLONAL RAT PHEOCHROMOCYTOMA (PC12) CELL LINE WITH MONOCLONAL ANTIBODIES (MAs). Virginia M.-Y. Lee* (SPON: A. Messing). Division of Neuropathology, Univ. of Pennsylvania School of Medicine, Phila., PA 19104.

RSN of newborn rats were cultured in the presence of nerve growth factor (NGF) for up to 3 weeks. PC12 cells grown in the absence of (PC12-) or in the presence of (PC12+) NGF were maintained in culture. Cytoskeletal proteins were extracted from RSN, PC12- and PC12+ cells at intervals in order to determine the sequence of NF and VF polypeptide expression as a function of time in culture. Accordingly, after SDS PAGE of the cytoskeletal extracts, the proteins were transferred electrophoretically to nitrocellulose paper and NF and VF polypeptides were detected using a selected group of MAs from a panel of 140 MAs specific for individual NF triplet proteins (200kD, 150kD or 68kD subunits) or VF protein. These MAs were prepared and their specificities defined as previously reported (P.N.A.S. USA 79:6089, 1982; J. Neuorochem. 42:25, 1984).

From the first to the tenth day in culture, RSN of newborn rats contained detectable levels of the two lower molecular weight NF subunits as well as VF protein; the 200kD NF subunit was not detected. At later time intervals, all three NF subunits could be detected in immunoblots and VF protein continued to be expressed by these cells. The immunoblot profile of NF and VF proteins in PC12- cells was identical to that just described for RSN grown in culture for the first ten days; no 200kD NF subunits were detectable. Growth of PC12 cells in NGF for up to three weeks did not alter this immunoblot profile.

These studies have characterized similarities and differences in the expression of NF subunits and VF protein in cultured RSN, PC12- and PC12+ cells. In RSN the expression of the 200kD NF subunit lags behind that of the other lower molecular weight NF triplet proteins while PC12- and PC12+ cells appear to express little or no 200kD protein at any time in culture. This data is in aggreement with our earlier hypothesis (Brain Res., 238:169, 1982; J. Neurosci., 1984, in press) that the synthesis and/or assembly of NF subunits in PC12 cells may be aberrant and that PC12 cells may be an attractive model system for studies of abnormal NF metabolism.

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260.14 EFFECTS OF LEAD ON NEUROBLASTOMA AND ASTROCYTE CELL CULTURES. E. Tiffany-Castiglioni,* A.J. Castiglioni, Jr., J. Zmudzki* and G.R. Bratton*. Dept. Veterinary Anatomy, Texas A&M University, College Station, TX 77843.

The effects of lead acetate on the viability and morphology of neural cell cultures was studied. The human neuroblastoma cell line SK-N-SH-SY5Y (SY5Y) and cultures of neonatal rat cerebral cortex enriched for astrocytes were used. Viability was measured by cell number (proliferation index) and trypan blue dye exclusion. In actively proliferating SY5Y cultures, treated one day after seeding with 10^{-4} to 10^{-6} M lead acetate, proliferation was inhibited, but dye exclusion was unchanged. After 3 days' exposure to lead-containing medium, the total cell number of treated cultures was about half that of control cultures. Simultaneous administration of thiamin (10^{-3}M or 10^{-4}M), which is used experimentally to treat lead intoxication, did not prevent this loss of proliferation. On the other hand, the toxic effects of lead were slightly enhanced. Thiamin itself had no effect on either parameter. SY5Y cells were shown to incorporate lead intracellularly by atomic absorption. Cultures treated with lead acetate (10^{-5}M) for three days were washed with EDTA in saline before analysis. Cultures incorporated 9 to 10 µg lead per 10^6 cells.

Astrocyte-enriched cell cultures were stained immunocytochemically for glial fibrillary acidic protein (GFAP). Qualitative examination of the slides showed that the number of GFAP-positive cells was decreased compared to controls. Total cell numbers, measured by counts of cell suspensions, were also reduced in actively proliferating cultures. Astrocytes exhibited a vesicular cytoplasm and lightly staining GFAP-positive small granules, unlike the fine filamentous network shown by control cells. Lead, therefore, did not induce a reactive response marked by brightly staining thick bundles of filaments, as we have observed in astrocytes treated with $FeCl_2$ or Al_2O_3.

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260.15 MACROPHAGE ATTACK ON STRESSED AND INJURED NEURONS IN CULTURE. C.R. Gardner*, M.H. Hightower*, and G.W. Gross, Dept. of Biology, Texas Woman's University, Denton, TX 76204. (SPON: J.F. Hines).

We are investigating cocultures of mammalian spinal cells and macrophages (MPs) to determine whether MPs interfere with neuronal recovery from process lesions. We have previously reported that neurons and MPs coexist in culture and that point lesions produced with laser microbeam surgery along neurites trigger MP chemotaxis resulting in phagocytosis of the affected process and occasionally of the cell body (Gardner and Gross. 1983. Soc. Neurosci. Abstr. 9:172). In our attempt to more accurately define the macrophage attack on neurons, we have initiated long term studies of this interaction and have employed a variety of staining techniques. So far, a combined Bodian-Nissl stain provides the best morphological data by selectively staining only neurons and MPs in great detail. Surprisingly, Wright's stain for blood elements and the assay for the presence of α-napthyl acetate esterase, a known MP marker enzyme, also stain only neurons and MPs without highlighting the glial carpet. Our data indicate that MPs attack only cells injured or stressed to the degree that cell death may be inevitable. These neurons are quickly (within 12-24 hrs.) and completely removed from cultures without damage to the adjacent healthy cells. Minor pH and osmolarity changes resulting in granular cell bodies do not elicit a MP response. Therefore, MPs could make a positive contribution to neuronal culture by cleaning debris and by removing slowly degenerating cells from established cultures. From the rapid (5 min. at 10-20µm) MP response, we consider it likely that small molecules, such as the oxidation products of arachidonic acid (known chemotactic agents), trigger MP chemotaxis.

The interesting question of whether MPs prevent a normal recovery from neurite amputation is being investigated by utilizing culture dishes in which separate adhesion areas are provided for dissociated mouse embryo spinal cells. Medium separation is maintained with a silicon gasket. Once the neuronal cultures are established, MPs are seeded on one side of the plate 3-5d prior to lesion experiments. After removal of the gasket and establishment of medium continuity, the same number and type of lesions are made in both regions of the culture and cells are followed for periods of 48 hrs. A comparative statistical study of cell recovery in these areas is being initiated.

260.16 INFLAMMATORY RESPONSES FOLLOWING EXPERIMENTAL SPINAL CORD INJURY: A DESTRUCTIVE EFFECT OF MACROPHAGES ON AXONS AND MYELIN? A. R. Blight, Depts. Neurosurg., Physiol. & Biophys., NYU Medical Center, New York, NY 10016.

Though it is clear that mechanical damage accounts for much of the functional loss following spinal cord injury, there is evidence to suggest that delayed processes of cellular disruption are responsible for a proportion of the ultimate dysfunction. The nature, time course and significance of these processes have been difficult to establish unequivocally, despite the fact that disparate experimental treatments reduce their effects (to small but significant extents).

This study was carried out in adult cats. They were anesthetized with sodium pentobarbital, the spinal cord exposed by laminectomy and injured with a standardized weight-drop contusion over the clamped T8 vertebra. After various periods of survival they were fixed by glutaraldehyde perfusion and the lesion site analyzed morphometrically (methods in Neuroscience, 10: 521, 1983).

The injury produced total destruction of the gray and extensive destruction of the white matter in the impact area. At more than 3 months after injury there were very low densities of myelinated axons (<100 per 10,000 μm^2), even in the outer 100 µm of the cord. There was some complete demyelination and a mean decrease in myelin thickness of 30-40%.

At 48 h after injury, intact axons were present in the sub-pial rim at densities above 100 per 10,000 μm^2. These showed little or no disturbance of myelin morphology. There was a variable, low density invasion of the lesion by leucocytes, with little sign of phagocytosis of cellular debris or of erythrocytes. At 8 days after injury, macrophages, packed with phagocytic vacuoles, comprised as much as 50% of the lesion volume. Many axons were completely demyelinated at this time.

Demyelination of surviving axons appears to be associated with the delayed inflammatory response and not to be a direct result of injury. Similarly, it appears that further loss of axons occurs in the presence of intense phagocytic activity. Initial electrophysiological data from isolated spinal tracts support this conclusion. Inflammatory cytolysis may be one of the most important delayed factors in the pathophysiology of spinal trauma.

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260.17 SUPEROXIDE DISMUTASE (SOD) INCREASES SURVIVAL OF CULTURED SYMPATHETIC NEURONS EXPOSED TO A BRIEF PERIOD OF STARVATION. J.C. Saez*, D.H. Hall, J.A. Kessler+, M.V.L. Bennett, and D.C. Spray, Depts. Neuroscience & Neurology+, Albert Einstein Coll. Med. Bronx, N.Y. 10461.

Deficient substrate supply (starvation) and hypoxia are two consequences of ischaemia. In vitro experiments allow separation of starvation from hypoxia, so that one can assess the contribution of each to the deleterious effects of ischaemia. Superior cervical ganglion neurons obtained from newborn rats were maintained in culture 3-4 wks in Ham's F12 nutrient solution plus 10% fetal calf serum. Starvation was induced by quickly washing the cells 4x with phosphate buffered saline (PBS); the cells were then held for 10 min in PBS and 95% air-5% CO_2 at 37°C and returned to nutrient medium. One hour later, survival (SR) determined by exclusion of trypan blue was 45.6 ± 4.2% (mean ± SD for 3 dishes). Preincubation (30 min) with SOD and 55 mM K^+ followed by 1 hr in medium containing SOD increased survival following starvation to 94.5 ± 5.2%. (Normal viability was not accurately determined but was near 99%). Resting potentials of surviving untreated, starved, and protected starved cells were 78 ± 7.5, 54 ± 4.5 and 67 ± 8.4 mV respectively. Little or no protection was seen of neurons preincubated 90 min with albumin (0.3 mM), with SOD inactivated by heating or with K^+ or SOD alone. In parallel experiments cells were preincubated (10 min) with nitro blue tetrazolium (1 mM) before starvation. Cells pretreated with K^+/SOD showed no color change after starvation. However, without K^+/SOD pretreatment or with K^+/ inactivated SOD pretreatment the somata became purple due to formazan precipitation, which suggests superoxide formation. High K^+ caused uptake of SOD as shown by I^{125} labelling of SOD and counts of treated tissue. High K^+ also caused uptake of horseradish peroxidase as shown by standard election microscopic methods. Thus SOD protection may be mediated intracellularly. No increase in survival was seen if K^+/SOD included 10 mM $MgCl_2$ or 50 µM TTX, which should reduce secretory and impulse activity respectively. These results support the hypothesis that free radicals, i.e. O_2^-, play a role in the cell damage caused by ischaemia (Demopoulus et al. Acta Neurol. Scand. Suppl. 64, 152 (1977). Supported in part by McKnight Development (DCS) and George Cotzius (JAK) awards and NIH grants NS 14830 and 20013.

260.18 CARBONIC ACID BUFFER BEHAVIOR IN BRAIN DURING COMPLETE ISCHEMIA. R.P. Kraig, W.A. Pulsinelli, & F. Plum. Dept. of Neurology, Cornell University Med. College, N.Y., N.Y. 10021

Excess H^+ accumulation in brain may cause irreversible injury. During complete ischemia the development of lowered $[Na^+]_o$ may prevent removal of excess cell H^+ by plasma membrane Na^+/H^+ antiport. Instead, only physicochemical buffers and plasma membrane Cl^-/HCO_3^- antiport can be expected to limit $[H^+]_i$ which would otherwise result from anaerobic glycolysis and ATP hydrolysis. Rat brain HCO_3^- stores should diminish in direct proportion to the consumption of other buffers with nearly equivalent ionization equilibrium constants as long as generated H^+, physicochemical buffers, and Cl^-/HCO_3^- antiport have access to the same tissue compartments. Accordingly, we simultaneously measured brain $[H^+]_o$, PCO_2, and lactate (LAC) during complete ischemia.

Rats were anesthetized with halothane, warmed to 37°C, and an artery and vein cannulated. Neocortices were exposed, superfused with Ringer, and impaled with H^+ (tridodecylamine) microelectrodes to a depth of 800μm. Adjacent surface PCO_2 was monitored with a MI-720 (Microelectrodes, Inc.) CO_2 electrode. Arterial pressure, pH, PO_2, PCO_2, and glucose were stabilized. Selected animals were given intravenous insulin (2-6units/kg U-100) or intraperitoneal 0.93M D-glucose (8.35 gm/kg) to alter brain carbohydrate stores and subsequent post ischemic LAC. Rats were killed by intravenous injection of 1 M KCl after successively longer intervals after the insulin/glucose injection. When brain $[H^+]_o$ and PCO_2 subsequently plateaued, heads were dropped into liquid nitrogen and LAC measured by enzyme fluorometric techniques. $[H^+]_o$ rose by 0.44±0.05pH (n=3) until LAC reached 13mmol/kg then $[H^+]_o$ rose by 1.00±0.04pH (n=7) and remained constant between 16-31mmol/kg LAC. Peak change in PCO_2 increased linearly with LAC until LAC reached 17mmol/kg then PCO_2 rose abruptly to 309±9mm Hg (n=7) and remained constant through 31mmol/kg LAC.

For $[H^+]_o$ to have remained constant for up to 13mmol/kg LAC while PCO_2 rose, either $[HCO_3^-]_o$ increased or a new buffer entered interstitial space. Since the peak change in PCO_2 did not increase after LAC reached 19mmol/kg, HCO_3^- stores were exhausted in those compartments where excess H^+ was generated. Tissue LAC up to 13mmol/kg during complete ischemia is correlated with selective loss of neurons while infarction occurs when LAC exceeds 16mmol/kg (Pulsinelli et al, 1982). We conclude that brain infarction from ischemia correlates with the loss of cellular HCO_3^- stores. (Supported by NS-19108, NS-03346, and a Teacher Investigator Award, NS-00767, and Rockefeller Clinical Scholar Award to R.P.K.)

260.19 ENDOTHELIAL CELL CHANGES IN EXPERIMENTAL HYPERTENSION AND DIABETES. P.A. Grady and O.R. Blaumanis*. Dept. of Neurology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201

Hypertension has been studied epidemiologically and found to be the single most important risk factor for the development of a stroke. However hyperglycemia or diabetes appears to be the major determinant in the morbidity and mortality of hypertensives who develop strokes. The underlying pathogenesis is not well understood. This study was designed to test the effect of the combination of risk factors hypertension and diabetes on brain blood vessels.

Adult, fully hypertensive spontaneously hypertensive rats were injected with streptozotocin (Sigma 40 mg/kg), a nitrosourea with a B cytotoxic effect on pancreatic cells and allowed to remain diabetic and hypertensive for one to four months. Blood pressure was monitored weekly using the indirect tail cuff method. Blood glucose levels were monitored weekly using the Harleco O-toluidine method. After periods of up to four months combination of diabetes and hypertension, animals were perfused through the heart with fixative. Major extracranial and intracranial vessels were removed and prepared for scanning electron microscopy (SEM).

After one month of combined hypertension and diabetes the extracranial carotid arteries showed an abnormal endothelial surface. SEM examination revealed endothelial surfaces with scattered areas of desquamation. These endothelial surfaces appeared "swollen" in appearance. The endothelial surfaces of brain vessels appeared pitted. Areas were observed in which endothelial cell orientation appeared random rather than the longitudinal orientation of cells normally seen.

After four months the internal carotid arteries of diabetic hypertensive rats appeared extensively "frothy" or swollen on the luminal surfaces. Endothelial surfaces of brain vessels were roughened and pitted. Frank endothelial cell injury was also observed in pial arteries.

In summary it appears that after even short periods of combined hypertension and diabetes result in structural changes in the luminal surfaces of extracranial and intracranial blood vessels which are different than those which occur with these risk factors singly and which have thrombogenic potential.

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TRANSMITTERS AND RECEPTORS IN DISEASE II

261.1 A QUANTITATIVE STUDY OF DOPAMINE AND SEROTONIN IN CEREBRAL ISCHEMIA BY HPLC. Hui Y. Bai*, Mang C. Yu and Z. Ye*. Dept. of Anatomy, New Jersey Medical School, Newark, N.J. 07103.

We reported previously (Neurosci. Abs. 9:973, 1983; Anat. Rec. 208:200A, 1984) that cerebral ischemia induced by carotid ligation caused pronounced morphological changes in the neuronal and neuroglial elements of the gerbil brain. Although there was slow recovery of structural integrity, the restitution was incomplete at 21 days after the ischemic insult. 2-deoxyglucose isotope uptake study also indicated reduced glucose utilization during the early phase (24 hrs) of ischemic insult, followed by an increase during later periods (80% of the control level by day 21 postischemia). In the present study, we reported on the status of two CNS neurotransmitters, dopamine and serotonin. Cerebral ischemia was induced in gerbils weighing 60-70g by ligating the right common carotid artery for 30-90 min. Of the 80 gerbils thus ligated, 36 developed neurological signs and only these were used in the study. The animals were killed at 3 hr, 4, 7 and 30 days postligation. Tissues from three brain regions, the frontal cortex, caudate nucleus and hippocampus of the right (ligated) and left (intact) hemispheres were dissected out and assayed quantitatively for dopamine and serotonin by high performance liquid chromatography with electrochemical detector (BAS, Inc., Indiana).

The results indicate significant changes in dopamine and serotonin contents in the caudate nucleus and hippocampus, and minor alteration in the frontal cortex. In the ischemic caudate nucleus, the values for dopamine at 3 hr, 4, 7 and 21 days were 70,50,76 and 74% of their control (intact) counterparts; the serotonin values were 70,45,38 and 56% of the controls. In the hippocampus, the dopamine level of the ischemic side was 50% at 3 hr, dropping sharply to 17 and 18 % at days 7 and 30, respectively. Serotonin was less severely affected, being 82,84,93 and 99% of controls at the respective survival periods. In the frontal cortex, both dopamine and serotonin levels remained about 90% of controls throughout the experimental periods. These data indicate considerable variations in neurotransmitter responses to ischemia in the three brain regions examined, with the caudate nucleus and hippocampus showing the greatest alteration to ischemic insult.

(Supported by Kimberly-McCurdy Foundation to M.C. Yu)

261.2 A RAT NEURAL END-POINT MODEL OF STROKE: PHYSOSTIGMINE REVERSAL OF HYPOXIA IMPAIRMENTS OF MEMORY, MOTIVATION, AND NEUROMUSCULAR PERFORMANCE. J.M. Ordy, Pennwalt Corp., Roch., NY 14623, G. Thomas, U. of Roch., Roch., NY 14624, and W. Dunlap, Tulane U., New Orleans, LA 70118.

Stroke is a leading cause of neurological impairments and the third leading cause of death in the United States. The incidence of stroke increases with age. Clinical approaches have focused on stroke in terms of cardiovascular lesions, thrombotic occlusions, and hypertension. Limits of neuronal resistance to anoxia are poorly understood. Decreases in the incidence of stroke have been attributed to improved diagnosis and treatment of hypertension. Recent evidence of greater neuronal resistance to anoxia than previously suspected has focused research on cellular causes of neurological deficits. Abnormalities of neurotransmitter metabolism, particularly acetylcholine, may mediate neurological impairments. Synthesis of neurotransmitters is critically dependent on molecular oxygen. Hypoxia and cholinergic antagonists produce similar neurological impairments. Physostigmine blocks cholinergically mediated impairments. Current animal models include the gerbil and the "Levine" rat model for assessment of morbidity and neuronal damage after carotid artery ligation. An animal model is needed in which ischemic-hypoxia and drug effects can be evaluated on specific neural end-points. The aims of this study were to evaluate the effects of hypoxic-hypoxia and physostigmine on trial-specific working memory, motivation, and neuromuscular performance of old Long-Evans rats. The memory test was conducted in a T-Maze adapted to present a spatial Delayed-Non-Matching-To-Sample (DMNTS) problem. It included trial-specific memory at 10, 90 and 180 sec. delays. Start, choice, and goal speeds served as indices of motivation and neuromuscular performance. Rats were placed on alternate days in a hypoxia chamber and exposed to a 96% N_2/4% O_2 atmosphere until loss of the righting reflex, or in a control chamber and exposed to a normoxic condition. Physostigmine was administered at 0.05 mg/kg, ip, 15 minutes prior to hypoxic-hypoxia. Under normoxic conditions, there was a significant decline in correct memory responses from 10 to 180 sec. delays ($P<0.01$). Neuromuscular performance did not differ significantly across 3 memory delays. Hypoxia produced a significant decrease in correct memory responses across 10, 90, and 180 sec. delays. Hypoxia also resulted in significant decreases in start, choice, and goal speeds ($P<0.01$). Physostigmine blocked the hypoxia effects on memory but not on motivation and neuromuscular performance. Results show usefulness of a rat neural end-point model of stroke.

261.3 WITHDRAWN

261.4 ROLE OF CATECHOLAMINE METABOLISM IN THE MATURATION OF INFARCTION IN ISCHEMIC STRIATUM. J. Weinberger and J. Nieves-Rosa* Department of Neurology, The Mount Sinai School of Medicine, New York, New York 10029.

The striatum is a region of brain that has a selective vulnerability to ischemic damage produced by unilateral carotid ligation in the Mongolian gerbil. The striatum contains a high concentration of dopamine (DA) nerve terminals, which have been shown to be more sensitive to ischemic damage than GABA, glutamate or serotonin terminals. The degeneration of the nerve terminals in ischemia is a delayed process. Uptake of DA proceeds normally for up to 8 hours, but is reduced to 15% of control at 16 hours (Weinberger, J., and Cohen, G., J Neurochem 38:963, 1982). Damage to nerve terminals was attenuated by depletion of catecholamines (CA) with alpha-methyl-para-tyrosine (AMPT) administered 6 hours prior to carotid ligation (Weinberger, J., Cohen, G., Ann Neurol 114:127 Abstract).

In order to determine if there was a relationship between CA metabolism and morphological evidence of ischemic damage, the brains of gerbils with stroke were examined for CA-derived fluorescence (CADF) by the Falck-Hillarp technique. Brains were removed from stroke animals at 0.5, 2, 4, 7 and 12 hours after carotid ligation, freeze dried at -35°C for 48 hours and incubated with paraformaldehyde to form fluorescent isoquinolines. Three stroke animals were studied at each time period. Specimens were examined with a Leitz transmission fluorescence microscope employing BG12 excitation filters and a K510 barrier filter. Morphological damage to neurons was assessed by the Nissl technique, employing a cresyl violet stain.

Two and 4 hours after stroke, there was depletion of CADF in the ischemic striatum, except in a midline border zone. In this border zone, CADF was present and there were pyknotic nuclei and vacuolization of the tissue on Nissl Stain. There were no degenerated neurons in the striatum void of CADF. Seven and 12 hours after stroke, there was restoration of CADF in the whole striatum, along with the appearance of pyknotic neurons and spongy degeneration of the tissue. The CADF could be removed with 0.1% sodium borohydride and reappeared when the tissue was reincubated with paraformaldehyde. Fluorescence was not seen in the striata of stroke animals when the tissue was incubated without paraformaldehyde.This temporal and spatial coincidence of CADF with onset of histologic ischemic damage suggests that CA metabolism is involved in the maturation of infarction in ischemic striatum.

261.5 DIFFERENTIAL COUPLING OF GABA-A AND GABA-B RECEPTORS TO THE NORADRENERGIC SYSTEM: IMPLICATIONS FOR A GABA-ERGIC ROLE IN DEPRESSION. P.D. Suzdak and G. Gianutsos. Section of Pharmacology and Toxicology, Univ. Connecticut, Storrs.

Norepinephrine (NE) and serotonin (5-HT) have been extensively studied as mediators of antidepressant (AD) drug action, while other neurotransmitters, such as GABA, have not recieved as much attention. Clinical and experimental data suggest a role for GABA in affective disorders, and this led us to investigate the role of GABA in the mechanism of action of AD drugs.Previously, the mixed GABA receptor (R) agonist progabide (Lloyd et al.,1983), THIP (GABA-A R agonist) and baclofen (GABA-B R agonist) (Suzdak and Gianutsos,1983) have been shown to decrease the sensitivity of Beta-adrenergic R after chronic administration, while AD drugs (e.g., imipramine and iprindole) have been shown to decrease the sensitivity of GABA-A R after chronic administration (Suzdak and Gianutsos, 1983), suggesting a functional coupling between the noradrenergic and GABA-ergic systems. To further test this hypothesis, the effect of various GABA-ergic drugs on NE turnover, using MHPG as a marker, was determined.In the cortex, both THIP and progabide dose dependently increased the formation of MHPG, and this effect was partially reversed by the GABA-A R antagonist bicuculline. In contrast, baclofen dose dependently decreased the formation of MHPG. Similiar results were obtained in the hippocampus.In an effort to determine how the GABA-A and GABA-B receptors are coupled to the noradrenergic system DSP4 (a neurotoxin selective for presynaptic noradrenergic neurons) was used. Mice were treated with DSP4, and three days latter chronic (14 day) treatment with either THIP, imipramine, baclofen,clenbuterol or saline began. In the cortex, DSP4 treatment resulted in a 135% increase in Beta-adrenergic R binding. Chronic treatment with either THIP or imipramine (which requires an intact noradrenergic neuron for its action) failed to alter the increase in Beta-adrenergic R produced by DSP4 treatment. But, chronic treatment with either baclofen or clenbuterol (a directed Beta-adrenergic R agonist) partially reversed the increase in Beta-adrenergic R produced by DSP4 treatment. These results suggest that the GABA-A R is coupled to the presynaptic noradrenergic neuron and functions by modulating NE release, while the GABA-B R is coupled to the post-synaptic noradrenergic neuron and functions through adenylate cyclase (Karbon and Enna, 1983), further suggesting an interaction between the noradrenergic and GABA-ergic systems that may be important for the mechanism of action of AD agents.

261.6 PLASMA INDOLES AND HORMONES FOLLOWING A 5-HYDROXYTRYPTOPHAN (5-HTP) OR TRYPTOPHAN (TRP) LOAD IN AFFECTIVE DISORDERS. T. Koyama*, M.T. Lowy, H.L. Jackman and H.Y. Meltzer, Univ. Chicago Pritzker Sch. Med., Dept. of Psychiatry, Chicago, IL 60637.

Previous studies from this and other laboratories have reported an increased cortisol (C) response to 5-HTP or a blunted prolactin (PRL) response to TRP in depressed patients (D) (Arch. Gen. Psychiatry 41, May, 1984). We have now examined plasma TRP, 5-HTP and 5-hydroxyindoleacetic acid (5-HIAA) levels using HPLC in plasma following p.o. 5-HTP or i.v. TRP.

The C response (peak-baseline over a 3 hr period) to D,L-5-HTP, 200 mg p.o., in 11 D (6.7 ± S.E. 2.9 μg/dl) was significantly greater than that of 13 normal controls (NC, -0.4 ± 1.8 μg/dl). Plasma 5-HTP responses were significantly greater in D (343 ± 30 ng/ml) than in the NC (258 ± 24 ng/ml, $p < 0.05$). Plasma 5-HIAA response in D (679 ± 34 ng/ml) was not significantly greater than in NC (581 ± 54 ng/ml, $t=1.48$, $df=22$, $p < 0.10$). The increments in 5-HTP, 5-HIAA and C were not significantly correlated in D or NC. These results suggest 5-HTP is absorbed more completely or metabolized more slowly by routes other than MAO in D than in NC. The greater C response in D after 5-HTP could be due, in part, to higher plasma 5-HTP levels but many other factors may influence this response.

L-TRP, 100 mg/kg i.v., was infused rapidly and plasma samples were obtained at -30 to +180 min via an indwelling catheter. Plasma TRP response was significantly less in D (N=13, - 173 ± S.E. 14.5 μg/ml) than in 10 NC (339 ± 42 μg/ml, $p < 0.001$). Plasma 5-HTP response in D (25.5 ± 3.7 ng/ml) and NC (22.3 ± 4.2 ng/ml) were not significantly different. Plasma 5-HIAA response in D (6.4 ± 0.8 ng/ml) were significantly lower than those of NC (44.5 ± 11.5 ng/ml). Plasma PRL response in D (9.4 ± 1.6 ng/ml) was not significantly different than that of NC (14.6 ± 3.6 ng/ml).

Thus, we did not confirm a previous report of a blunted PRL response to TRP in depressed patients although our results are in the same direction. The lower TRP levels in D may contribute to the smaller PRL response; it could be due to more rapid conversion of TRP via the pyrrolase pathway but many other factors are possible. The markedly lower 5-HIAA levels in D suggests that serotonin (5-HT) formed from 5-HTP is not readily metabolized in these patients by MAO. This was not observed with oral 5-HTP which produced 10-15 fold higher 5-HTP levels. It is possible that the affinity of MAO for 5-HT is decreased or that neuronal 5-HT uptake is less in D.

261.7 CHANGES IN NEUROTRANSMITTER RECEPTOR BINDING IN THE HIPPOCAMPAL FORMATION OF PATIENTS DYING WITH SENILE DEMENTIA OF THE ALZHEIMER TYPE: A QUANTITATIVE AUTORADIOGRAPHIC STUDY. A. Probst*, J.M. Palacios and R. Cortes*. Inst. of Pathology, Dept. of Neuropath., Univ. of Basel and Preclin.Res., Pharm.Div., Sandoz Ltd. Basel, CH-4002.

Alterations in the number of receptors for neurotransmitters and drugs acting on these receptors have been described in several pathologies of the CNS. In particular, variations in the number of some receptor binding sites have been reported in senile dementia of the Alzheimer type (SDAT) (Reynolds G.P. et al., Neurosci.Lett. 44: 47-51 (1984). We have now used quantitative light microscopic autoradiographic techniques to analyze these changes with a higher anatomical resolution. Receptors for acetylcholine, serotonin, noradrenaline, GABA, benzodiazepines, opiates and glycine were labeled in vitro in microtome sections from postmortem material using several ^{3}H-ligands. Receptor binding densities were measured using computer assisted microdensitometric techniques. Muscarinic cholinergic, serotonin, GABA$_A$ and benzodiazepines receptor binding (labeled with ^{3}H-N-methylscopolamine, ^{3}H-LSD, ^{3}H-muscimol and ^{3}H-flunitrazepam respectively), was found to be decreased in the hippocampal formation in some cases of senile dementia but not in all cases. Characteristics of the cases showing a decrease in receptor binding were 1) severe neuronal loss and frequent extracellular remnants of neurofibrillary tangles 2) few senile plaques. Both, neuronal loss and the decrease in binding sites predominated in the CA_1 region of the hippocampus and the subiculum. In contrast other cases showing a large number of senile plaques, but, less severe neuronal loss presented normal densities of receptor sites. In fact, the neuropil of the senile plaques appeared, at the resolution of this technique, as rich in receptors as other areas without plaques.

In conclusion using light microscopic autoradiography we have observed localized losses of neurotransmitter receptor binding sites in the hippocampus of some patients dying with SDAT. These losses are not specific for any receptor studied so far and appear to be related to concomitant neuronal losses. While the pathological significance of these receptor losses is unclear at the present time, it could be speculated that they reflect different stages of the disease.

261.8 LOSS OF SOMATA IN NUCLEUS BASALIS IS CORRELATED POSITIVELY WITH MAGNITUDE OF DEMENTIA, SEVERITY OF PATHOLOGY, AND AGE OF ONSET OF ALZHEIMER'S DISEASE. R. W. Jacobs, N. Farivar* and L. L. Butcher. Brain Research Institute and Dept. of Psychology, University of California, Los Angeles, CA 90024.

Providing a substantial proportion of the cholinergic innervation of the non-striatal telencephalon, the nucleus basalis and associated basal forebrain structures have been implicated in the pathology and clinical manifestations of Alzheimer's disease (AD) and senile dementia of the Alzheimer type (SDAT). Indeed, significant loss of these cells has been observed in AD/SDAT (Whitehouse et al., Science, 215:1237, 1982), which accounts for the earlier findings of decreased cholinergic indices (e.g., acetylcholinesterase {AChE}, choline-O-acetyltransferase {ChAT}, acetylcholine synthesis) in these dementing disorders (for review, see Bartus et al., Science, 217: 408, 1982). In addition, Perry and Perry (Brit. Med. J., 2: 1457, 1978) have reported that decreased cortical ChAT is correlated with decreased intellectual functioning and increased plaque counts in AD/SDAT. In this report, we present additional aspects of the role of nucleus basalis in AD/SDAT. A preliminary analysis of its involvement in other dementing disorders is attempted also.

Postmortem brains (11 cases of AD/SDAT, 4 with other dementing illnesses, 4 aged controls, and 6 young controls) were received from the National Neurological Research Brain Bank (Wadsworth VA Hospital; Los Angeles, CA) and the St. Paul Ramsey Medical Center (St. Paul, MN). Successive 60 μm thick sections were prepared and stained sequentially for Nissl substance, AChE, NADH-diaphorase, neurofibrils and plaques (Bodian procedure), and amyloid (Congo Red procedure). Cell counts in nucleus basalis were performed blind by two independent raters.

Compared to age-matched controls, AD/SDAT patients displayed a 50% loss of cells in nucleus basalis. Within the AD/SDAT population, there was a positive correlation between nucleus basalis neuron decrements and the severity of clinically assessed dementia ($r = 0.88$, $p < 0.01$) and the degree of neuropathology ($r = 0.92$, $p < 0.01$). In addition, AD/SDAT patients developing dementia at an earlier age showed greater loss of nucleus basalis cells at autopsy. In patients suffering from other dementing disorders the relationship between nucleus basalis involvement and the pathologic and clinical profile was inconsistent.

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261.9 ALTERED PATTERNS OF L-[^{3}H]GLUTAMATE BINDING IN ALZHEIMER'S DISEASE AND HUNTINGTON'S DISEASE. A. B. Young, J. T. Greenamyre, J.B. Penney, C.J. D'Amato, S.P. Hicks and I. Shoulson. Univ. of Michigan, Depts. of Neurology and Pathology, 1103 E. Huron Ann Arbor, MI 48104 and Dept. of Neurology, Univ. of Rochester, Rochester, NY 14642.

Glutamate (GLU) is believed to be a major excitatory neurotransmitter of both cortical-cortical and corticofugal pathways. Using an autoradiographic technique to label putative GLU receptors with L-[^{3}H]glutamate, we have previously shown an excellent correlation between the location of GLU binding sites and terminal fields of glutamatergic pathways (Greenamyre et al., J.Neurosci., In press). [^{3}H]-glutamate binding was measured by this technique in whole coronal sections of brains from 5 patients dying of non-neurologic disease, 5 patients dying of senile dementia of the Alzheimer type (SDAT) and 4 patients dying of Huntington's disease (HD). In SDAT brains, GLU binding in layers 1 & 2 of cortex was decreased by approximately 45% and in layers 5 & 6 by about 35% compared to control brains ($p < 0.01$). In the same brains, GLU binding in the caudate, putamen and claustrum was normal relative to control cases. The changes in SDAT brains represent a decrease in the maximum number of GLU binding sites with no apparent change in the affinity of binding; there were no significant changes in cortical binding of other neurotransmitter receptor ligands in these brains. In the brains of HD cases, GLU binding was decreased by 68% in caudate ($p<0.001$) and by 67% in putamen ($p<0.01$) as compared to control cases; binding of other neurotransmitter ligands was also decreased in the caudate and putamen as has been described previously (Penney, J.B. and Young, A.B., Neurology 32(12). Binding in cortex of HD brains did not differ significantly from controls.

This information provides the first autoradiographic visualization of L-[^{3}H]glutamate binding in human brain and the first demonstration of glutamate receptor changes in human neurologic diseases.

Supported by the Arbogast Foundation.

261.10 CENTRAL TRYPTAMINE TURNOVER IN DEPRESSION, SCHIZOPHRENIA, AND ANOREXIA: MEASUREMENT OF INDOLEACETIC ACID IN CEREBROSPINAL FLUID. George M. Anderson,*[1], Robert H. Gerner, *[3], Bennett A. Shaywitz, [2], Donald J. Cohen,*[1], & L.Fairbanks, *[3]. Child Study Center [1] and Department of Neurology [2], Yale University Medical School, 333 Cedar Street, New Haven, Ct. 06510, and Department of Psychiatry [3], UCLA Center for Health Sciences, Los Angeles, Ca. 90024.

There has been a continuing interest in the possible role of the trace amine tryptamine in the etiology of neuropsychiatric disorders. It has been well established that the major central nervous system metabolite of tryptamine is indole-3-acetic acid (IAA) and that IAA found in cerebrospinal fluid (CSF) originates from the brain. We have, therefore, examined CSF levels of IAA in a large group of normals and in several patient populations. No differences in CSF IAA levels (ng/ml,mean ± SEM) were observed between normals (4.39 ± 0.37, N=36), anoretics (4.40 ± 0.42, N= 35), schizophrenics (4.06 ± 0.05, N=17), or depressives (5.23 ± 0.49, N=39). A significant elevation ($p=.05$) was found in the subgroup of retarded depressives (RDC criteria) where levels of 5.90 ± 0.80 (N=19) were observed. An age effect ($r=+.39$, $p=.02$, N=36) was observed in normals; however, IAA was not related to either height or weight. When the normal group was divided into subjects over and under 40 years of age, mean IAA levels of 6.35 ± 1.17 and 4.07 ± 0.37 ng/ml were observed, respectively. Age was not correlated with CSF IAA in the under-40 group ($r= -.02$); however, in the over-40 group, a positive correlation was observed ($r= +.78$, $p=.05$, N=5). An even more marked age-difference was seen in normal neonatal subjects where average IAA levels of 44.4 ng/ml (n=17) were observed. IAA tended to be higher in females in the groups studied; this difference was significant ($p=.05$) when all diagnostic groups were combined (females: 5.26 ± 0.60, N=71; males: 4.20 ± 0.41, N=66). In general, the results indicate that central tryptamine turnover is not altered in the disorders studied. The cause and functional significance of the slight elevation of CSF IAA seen in retarded depression is not clear. In normals, CSF IAA appears to decline markedly in early life, stablize from ~15-40 years of age,then increase gradually.

261.11 RESPONSES OF THE PIGMENTED RABBIT RETINA TO N-METHYL-4-PHENYL 1,2,3,6-TETRAHYDROPYRIDINE, AN INDUCER OF PARKINSONISM IN MAN AND MONKEYS. C.G. Wong*, G.S. Tucker, and D.I.Hamasaki*. Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, FL. 33101.

Short term administration of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (NMPTP) in monkeys and man induces symptoms characteristic of Parkinsonism. NMPTP appears to act by selective destruction of dopaminergic neurons in the substantia nigra. The neurotransmitter dopamine (DA) has been identified in certain amacrine cells in the rabbit retina. Since NMPTP affects dopaminergic neurons in the CNS, this study was done to determine the neurochemical,electrophysiological, and morphological responses of the rabbit retina to NMPTP.

Young pigmented rabbits were injected intravenously with 2mg/kg NMPTP. DOPA, DA, and DOPAC levels were measured by reverse phase ion pair HPLC with electrochemical detection. One hr. after NMPTP injection, DOPAC levels were not detectable; DOPA levels had decreased by 26% ($t=2.03$, $df=6$, $p<0.05$) and DA levels remained unchanged ($t=0.03$, $df=8$). After 5 hrs., DOPA could not be detected while DA decreased by 30% ($t=2.63$, $df=6$, $p<0.025$). Two days after chronic treatment with NMPTP (2mg/kg/day for 12 to 15 days), DOPA increased by 2-fold ($t=1.16$, $df=6$, $p<0.01$) while DA was similar to controls ($t=0.18$, $df=6$).

Following IV injection of NMPTP, the implicit time of the electroretinogram in 2 of 5 animals increased by 6 msec. after 4 hrs. The amplitude of the b-wave decreased by 31.5% ±6.5(SEM) ($t=4.84$, $df=8$, $p<0.001$). Depression of the oscillatory potentials on the descending limb of the b-wave occur at that time. These OPs are thought to originate from amacrine cells while both amacrine and bipolar cells contribute to the b-wave.

Toluidine blue-stained retinas from normal, acute, and chronic specimens had normal morphology. These retinas were ultrastructurally normal except for the occurrence of a paracrystalline structure in the nucleus of some amacrine and bipolar cells of chronic animals. These structures appear similar morphologically to inclusions reported in aging rat and mouse brains. The incidence of Parkinsonism which is accompanied by selective dopamine deficiency in the CNS increases with aging. These results suggest that dopaminergic neurons in the rabbit retina may provide a model system for studies on Parkinsonism in addition to expanding the understanding of the functional characteristics of retinal neurons.

261.12 OPIATE BINDING ACTIVITY IN THE MEDULLA OF SUDDEN INFANT DEATH SYNDROME (SIDS) VICTIMS S.S.Stensaas, R.R.Dean*, and J.K.Wamsley., Depts. of Path., Pharm., and Psych., Sch. of Med., Univ. of Utah, Salt Lake City, Utah 84132

Recently Kuich and Zimmerman (Med. Hypoth 7:1231, 1981) hypothesized that excessive endorphin activity may be involved in the etiology of Sudden Infant Death Syndrome (SIDS). Orlowski and Lonsdale (Clev. Clin. Quart. 49:87, 1982) found elevated endorphin levels in the CSF of a number of near-miss SIDS victims adding support to the theory. The present study was undertaken to look for differences in opioid receptor density in brainstem respiratory areas of SIDS victims compared to controls. Brain tissues from SIDS victims and control children were employed in the study. Six different age groups were examined: 3, 8, 9, 12, 16 and 20 weeks. Tissues were sex, age, and postmortem time matched. Groups consisted of 1 control vs. 1-4 SIDS brains. This initial study examined rostral, middle and caudal medulla. Specifically the nucleus of the tractus solitarius, dorsal motor nucleus of the vagus and the spinal trigeminal nucleus.

Transverse sections (10μ thick) were thaw-mounted on gelatin coated slides. Slides were pre-incubated in buffer containing a high concentration of GTP and NaCl to dissociate any endogenous ligand. Sections were incubated in tritiated dihydromorphine (4nM, N.E.N. 80.3 Ci/mM). Anatomically adjacent tissue sections were incubated under similar conditions with the added presence of naloxone (1 μM) as a displacer to determine regions of non-specific binding. After the incubation period, the sections were rinsed in ice cold buffer to wash away any unbound ligand. Autoradiograms were obtained by apposing sections to tritium sensitive film (LKB Ultrofilm) and exposed for three months. Four autoradiograms from each brain were analyzed at each level (caudal, middle and rostral medulla) using computer-assisted microdensitometry. Large variations between groups or between left-right sides of the same brain were seen. This variability contributed to the failure of the preliminary data to show any statistically significant differences in opioid binding in SIDS tissue vs. control. However, by standardizing the density measurements against readings taken from the inferior olive (to minimize the variability in the data due to differences in postmortem time and tissue thickness), a trend toward elevated opioid receptor densities in the SIDS tissues vs. controls was suggested.

261.13 **LOCALIZATION OF SUBSTANCE P, 5HT AND TRH-LIKE IMMUNOREACTIVITY IN MOUSE SPINAL CORD FOLLOWING MOTOR NEURON DAMAGE.** E.M. Zimmermann*, J.A. Coffield*, V. Miletic, M.J. Hoffert, B.R. Brooks. Neurology Dept., Univ. of Wisconsin Med. Sch. & Dept. of Struct. & Funct. Sci., Univ. of Wisconsin Sch. of Vet. Med., Madison, WI 53792.

We used a modified peroxidase-antiperoxidase technique (J. Neurosci. 2:1660, 1982) to localize Substance P (SubP), 5-hydroxytryptamine (5HT), and thyrotropin-releasing hormone (TRH) -like immunoreactivity (LI) in the spinal cord of normal mice (n=6) and mice with motor neuron damage (n=8). Motor neuron damage was induced by innoculation with murine neurotropic retrovirus or by proximal sciatic neurectomy. Five of the virus innoculated animals also received daily injections of TRH for 16 weeks.

We found that the distribution of SubP-LI and 5HT-LI in normal mouse spinal cord supported previous observations. SubP-LI was found in fibers throughout the gray matter with the heaviest concentration in laminae I & X. Fibers with 5HT-LI were also found throughout the gray matter with the heaviest concentration in lamina I and surrounding motor neurons in lamina IX. TRH-LI was found in varicosities surrounding motor neurons in laminae VIII and IX. No difference in distribution of neuropeptide-LI was observed between cervical and lumbar spinal cord sections in normal mice.

		Motor Neuron Damage		
Neuro-Transmitters	Sham Neurectomy	Sciatic Neurectomy	Virus Innoculation -TRH	+TRH
SubP	N	V	V	V
5HT	N	↓F,↑#	↓F,↑#	N
TRH	N	↑S	↑S	N

V= Variable change in SubP-LI in laminae I & X
↓F= Marked decrease in number of fibers containing 5HT-LI in lamina VII
↑#= Probable increased number of varicosities with 5HT-LI around motor neurons in laminae VIII and IX
↑S= Increased size of varicosities (diameter: normal ≤ 2.1 μm; affected ≤3.3 μm) containing TRH-LI around motor neurons in laminae VIII and IX
N= Immunoreactivity similar to normal mouse spinal cord

In conclusion, motor neuron damage induced by sciatic neurectomy or retrovirus infection was associated with redistribution of fibers containing 5HT and TRH-LI. Treatment with TRH normalized the redistribution of fibers containing 5HT and TRH-LI associated with retrovirus induced motor neuron damage.
(Supported by grants from ALSSOA and MDA.)

TRANSMITTERS AND RECEPTORS IN DISEASE III

262.1 BRAIN DAMAGE RESULTING FROM INTRAAMYGDALOID ADMINISTRATION OF D-TUBOCURARINE. J. Labruyere*, J.W. Olney and M.T. Price, (SPON: S.B.Guze). Washington Univ Schl Med, St. Louis, MO.

Recently we injected a series of muscarinic cholinergic agonists and cholinesterase inhibitors into the rat amygdala (basolateral nucleus) and found that some agents in each class induced sustained limbic seizures and a pattern of disseminated brain damage similar to that we have observed in association with seizures induced by intraamygdaloid injections of kainic acid or folic acid. The seizure-brain damage syndrome (SBD) induced by cholinergic agents is prevented by pretreatment with large doses of atropine (150 mg/kg sc). One of the most potent and consistently effective cholinergic agents studied was neostigmine which predictably induced a SBD syndrome from injection of 4 nmol into the amygdala. Since cholinesterase inhibition is the primary mode of neostigmine action, the SBD syndrome induced by neostigmine can presumably be attributed to excessive activation of cholinergic receptors by endogenous ACh. However, if this is so, both muscarinic and nicotinic receptors might be playing a role. To clarify this point, we injected D-tubocurarine (D-tubo), a nicotinic receptor antagonist (as tested in the periphery) together with neostigmine into the rat basolateral amygdala. This resulted not in suppression of any component of the reaction as we anticipated it might, but in a striking augmentation. In subsequent testing of D-tubo by itself it became evident that it is a potent inducer of the same SBD syndrome observed in neostigmine-treated rats. This syndrome occurs predictably when relatively low doses (1-5nmol) of D-tubo are injected into the basolateral amygdala. One possible interpretation of this finding would be that D-tubo, by inhibiting inhibitory nicotinic receptors is disinhibiting amygdaloid pathways that are potential generators of limbic seizure activity. A puzzling additional feature of our findings, however, is that higher doses of D-tubo (30-60 nmol) cause not only severe distant disseminated (presumable seizure-mediated) brain damage but local damage at the injection site which appears to affect essentially all local components including axons, dendrites, neuronal cell bodies and glia. This is in contrast to other cholinergic agents which do not induce local damage even when administered in high doses. It suggests that D-tubo may act by more than one mechanism, none of which is adequately understood at present. (Supported by Research Scientist Award MH30894 to JWO.)

262.2 ELECTRICALLY STIMULATED DOPAMINE RELEASE FROM SLICES OF THE NUCLEUS ACCUMBENS: EFFECTS OF HALOPERIDOL AND CLOZAPINE TREATMENT. D.R. Compton and K.M. Johnson. Dept. of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550.

Excessive release of dopamine (DA) is believed to be a major factor involved in the manifestation of symptoms in schizophrenic patients. One problem with the DA theory is the delayed onset (2-4 weeks) of therapeutic efficacy with neuroleptic treatment, while DA receptor antagonism is immediate. It has recently been suggested, based on experiments involving chronic neuroleptic treatment of rats, that delayed therapeutic efficacy may be related to a delayed decrease in the number of spontaneously active A10, DA neurons. We hypothesized that decreased neuronal activity would correlate to decreased DA release.

Two hours after acute or chronic (1/day x 21 days) administration of either saline, haloperidol (HAL) (0.5 mg/kg), clozapine (CZP) (20 mg/kg), male Sprague-Dawley rats were decapitated and 0.3 mm slices of the nucleus accumbens (an A10 projection field) were prepared. The slices were loaded with 3H-DA in the presence of pargyline for 30 min and then superfused with buffer containing a DA uptake inhibitor (nomifensine). Fractional 3H-DA release was evaluated under spontaneous efflux and electrical field stimulation conditions (15V, 2 msec, 60 pulses, at 1,5, and 10 Hz). Acute HAL, but not CZP, enhanced electrically stimulated release by 102,126 and 117% at 1, 5, and 10 Hz respectively. No acute effect on spontaneous efflux was observed. Chronic administration of neither agent altered either spontaneous or electrically stimulated release of 3H-DA.

Although there is tolerance development to the acute effects of HAL, this data indicates that there is no change in electrically stimulated DA release after chronic treatment with these two drugs. If therapeutic efficacy is related to decreased DA release, then this decrease is due to altered impulse flow from the cell body, or the changes in the release process were masked by the presence of nomifensine.

262.3 STUDIES ON THE MECHANISM OF SEROTONIN$_2$ RECEPTOR DOWN-REGULATION BY ANTIDEPRESSANTS. J.A. Scott* and F.T. Crews, Dept. of Pharmacology, University of Florida School of Medicine, Gainesville, FL 32610.

The therapeutic response to antidepressants is believed to be due to a progressive down-regulation of several receptor subtypes including Beta-adrenergic and $5HT_2$ receptors. Down-regulation of β receptors by antidepressants appears to be secondary to increased stimulation by norepinephrine. However, the mechanisms of the antidepressant-induced down-regulation of $5HT_2$ receptors is not known. We have investigated the role of serotonin in the $5HT_2$ receptor down-regulation using two experimental approaches. In one set of experiments, rats were treated i.p. with reserpine (5 mg/kg/day) for 4 days, which is sufficient to deplete central stores of both norepinephrine and serotonin. Receptor binding to membranes from the cerebral cortex and hippocampus was determined using [^{3}H]dihydroalprenolol for β receptors and [^{3}H]spiperone for $5HT_2$ receptors. Reserpine treatment significantly increased β receptor binding in both regions, but did not change $5HT_2$ receptor binding in either brain region. In another set of experiments, rats were treated by i.p. injection for 3 weeks with the antidepressant amitriptyline (10 mg/kg/day) alone or in combination with the $5HT_2$ receptor antagonist nefazadone (50 mg/kg/day). Beta and $5HT_2$ receptor binding was determined 24 hours after the last drug dose as described previously. Amitriptyline treatment significantly decreased both β and $5HT_2$ receptor binding in the cerebral cortex. The coadministration of nefazadone had no significant effect on the down-regulation of $5HT_2$ receptors by amitriptyline. Therefore, the down-regulation of $5HT_2$ receptors by antidepressants does not appear to be mediated through changes in the availability of serotonin. To investigate a possible β-$5HT_2$ interaction in the down-regulation of $5HT_2$ receptors by antidepressants, we treated rats i.p. with amitriptyline (10 mg/kg/day) alone or in combination with the β receptor antagonist propranolol (10 mg/kg/day) for 3 weeks. Amitriptyline significantly decreased both β and $5HT_2$ receptor binding. Coadministration of propranolol abolished the down-regulation of β receptors by amitriptyline, but had no effect on the down-regulation of $5HT_2$ receptors. Therefore, the antidepressant-induced down-regulation of $5HT_2$ receptors does not appear to be mediated through a β-$5HT_2$ receptor interaction.

262.4 ION-EXCHANGE/FLUOROMETRIC ASSAY WITH ENHANCED RESOLUTION FOR GABA IN CSF. Thomas N. Ferraro and Theodore A. Hare. Dept. of Pharmacology, Thomas Jefferson Univ., Philadelphia, PA 19107

GABA in human CSF has been quantified by a number of techniques including radioreceptor, gas chromatographic-mass spectroscopic and ion-exchange/fluorometric (I-E/F) assays. Although the radioreceptor assay is most widely used, the I-E/F method has been documented to have both superior precision and specificity (Hare, Mol. Cell. Biochem. 39: 297, 1981; Ferraro et al., J. Neurochem. 41: 1057, 1983). A modification of existing I-E/F procedures for GABA determination in our laboratory has permitted even greater specificity than reported previously (Hare and Manyam, Anal. Biochem. 101: 349, 1980). The modified procedure employs a 1.0 m rather than a 50 cm microbore ion-exchange column (2 mm i.d.) which is eluted isocratically with a 0.4 N lithium citrate buffer (pH 4.40). Retention time of GABA is increased from 25 to 80 min; nonetheless, the design of the triple-1.0 m column GABAlyzer with automatic sample application allows 48 GABA analyses in a 24 hr period. In a comparative study, specimens of human lumbar CSF (n=10), collected under standardized conditions as part of another investigation, were deproteinized with one-third volume 20% aqueous sulfosalicylic acid and analyzed in triplicate on both the 50 cm column and 1.0 m column systems. A significant correlation ($r=.96$, $p < .001$) was found between the two systems with lower values given by the 1.0 m columns (CSF GABA levels ($\bar{X}$±SD): 126±37 pm/ml vs 83±25 pm/ml, $p < .001$, paired t-test). These results indicate improved resolution of GABA from the numerous unknown substances in CSF which exhibit similar chromatographic behavior on ion-exchange HPLC systems and suggest that compared to other reported methods, I-E/F techniques employing longer columns may offer a more accurate means of measuring GABA in CSF.

262.5 HIGH AND LOW AFFINITY [3H]IMIPRAMINE BINDING IN BRAIN OF FAWN-HOODED RATS. E. Tobach*, J.R. Ieni, S.R. Zukin, and G. Barr*. Dept. of Psychiatry, Albert Einstein Col. of Med., Bronx, N.Y. 10461 and American Museum of Natural History, N.Y., N.Y. 10016.

The Fawn-Hooded rat is a strain deficient in platelet storage of 5-HT. In addition to this deficit, Dumbrille-Ross and Tang (1981) reported that Fawn-Hooded rats have an absence of both platelet and cortical high-affinity [3H]imipramine binding sites. However, Arora et al. (1983) did find high-affinity [3H]imipramine binding in Fawn-Hooded rats, but cortical and platelet densities were significantly lower than those observed in Sprague-Dawley rats. In contrast, Murray and co-workers (1983) found that both Fawn-Hooded and Fawn-Hooded-cross rats had similar densities of [3H]imipramine binding sites whether on blood platelets or in cerebral cortex when compared to Sprague-Dawley rats. Only when compared to a genetically more appropriate control, the NBR-cross strain, did the cerebral cortical membranes of Fawn-Hooded rats show significantly lower densities in high-affinity [3H]imipramine binding. The focus of the present study was to determine whether Fawn-Hooded rats possess both high- and low-affinity [3H]imipramine binding sites and compare the densities of binding sites to rats of another strain.

Brains from Fawn-Hooded and Long-Evans strains were homogenized in 50 mM TRIS buffer containing 120 mM NaCl and 5 mM KCl (pH = 7.5 at 7° C). Homogenates were incubated (1 hr at 0-4° C) with 0.1 to 500 nM [3H]imipramine in the presence of 100 μM desipramine or buffer. Binding was terminated by rapid filtration through GF/B filters. Data analysis was performed using the LIGAND program (Munson, 1979; Teicher, 1982).

Non-linear scatchard analysis showed that data from both the Fawn-Hooded and Long-Evans strains fit a two-site model. The Fawn-Hooded rats showed apparent Kd values of 7.0 and 1173 nM and Bmax values of 661 fmol and 9.635 pmol/mg protein for the high- and low-affinity binding sites, respectively. Long-Evans rats showed apparent Kd values of 4.5 and 898 nM and Bmax values of 376 fmol and 8.232 pmol/mg protein for the high- and low-affinity binding sites, respectively.

The results of the present study suggest that Fawn-Hooded and Long-Evans rats possess similar whole brain densities of high-affinity [3H]imipramine binding sites. In addition, Fawn-Hooded rats possess a low-affinity component similar to that found in rat brain and human platelets.

262.6 INCREASED ACETYLCHOLINE RELEASE FROM BRAIN SLICES OF CATS WITH Gm_1 GANGLIOSIDOSIS. R.S. Jope* and H.J. Baker* (SPON: L. Tolbert). Depts. of Pharmacology and Comparative Medicine, University of Alabama in Birmingham Medical Center, Birmingham, AL 35294.

Severe progressive central nervous system dysfunction is a prominent feature of many lysosomal storage diseases including the gangliosidoses. Although specific defects in lysosomal biochemistry have been characterized in these diseases, the pathogenetic mechanisms of neuronal dysfunction are not known. In this study we utilized a well characterized feline model of Gm_1 gangliosidosis (Baker, H.J. et al., Vet. Path. 16:635, 1979) to evaluate cholinergic metabolism in brain slices using gas chromatography-mass spectrometry to quantitate acetylcholine.

The K^+-stimulated (50 mM) release of acetylcholine was significantly higher in both cortical slices (165% of controls) and hippocampal slices (142% of controls) of 5 cats with Gm_1 gangliosidosis compared with age matched normal cats. Resting (4.75 mM K^+) release of acetylcholine was not altered. The resting tissue concentration of acetylcholine also was higher in affected cats (130% of controls). High affinity choline transport measured in cortical synaptosomes of affected cats was 131% of control values.

These data indicate that synthesis and release of acetylcholine are increased in two brain regions of cats with Gm_1 gangliosidosis suggesting that abnormal neurotransmitter function may contribute to the pathogenesis of neuronal dysfunction in this disease.

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262.7 INCREASED ANXIOGENIC EFFECTS OF CAFFEINE IN PANIC DISORDER PATIENTS. D.S.Charney* and G.R.Heninger, (SPON: M.Bowers). Dept. Psychiatry, Yale Univ. Sch. of Med., New Haven, Ct.

In higher doses, caffeine has been demonstrated to produce symptoms of anxiety and nervousness in healthy subjects and psychiatric patients. The molecular mechanism responsible for these actions in humans has not been definitively established. However, there is behavioral, electrophysiological, and biochemical evidence indicating the most likely mechanism is antagonism of central adenosine receptors. The purpose of the present investigation was to evaluate the effects of caffeine on behavior, norepinephrine turnover, somatic symptoms, and blood pressure in healthy subjects and patients meeting DSM III criteria for Agoraphobia with panic attacks or Panic Disorder.

Methods: Patients (N=21) and healthy subjects (N=17) were closely matched by age and sex. Both groups had not received psychotropic drugs for at least 21 days and had been maintained on a caffeine free diet for at least 14 days prior to the study. Placebo was given on the first test day and caffeine (10 mg/kg) on the second. Blood samples, behavioral ratings, and blood pressure were obtained before and at several time points following drug administration. Plasma 3-methoxy-4-hydroxyphenylethleneglycol (MHPG) was used as a measure of norepinephrine turnover and was determined by gas chromatography and mass spectrometry. Visual analogue scales and a physical symptom scale were used to evaluate changes in behavior and somatic symptoms.

Results: Caffeine significantly increased self ratings of nervous and anxious in both the healthy subjects and patients. However, in the patients the peak increases in nervous and anxiety were more than double those observed in the healthy subjects ($p<.02$). The patients reported larger increases in anorexia ($p<.05$), nausea ($p<.01$) and restlessness ($p<.05$). The peak increase in sitting diastolic blood pressure was also greater in the patients ($p<.05$). Caffeine did not alter plasma MHPG levels in the healthy subjects or the patients.

Implications: The increased sensitivity of panic anxiety patients to the anxiogenic effects of caffeine suggest that these patients have abnormalities in adenosine receptor function. However, since adenosine has inhibitory actions on numerous neuronal systems in brain, it is possible that functional alterations of these systems are also related to the present results. From a clinical standpoint, panic anxiety patients should avoid caffeine containing foods and beverages.

262.8 ANALOG SPECIFICITY OF THYROTROPIN-RELEASING HORMONE RECEPTOR IN THE CNS: POSSIBLE CLINICAL IMPLICATIONS. E.F. Hawkins and W. K. Engel. USC Neuromuscular Center, Los Angeles, CA 90017

In patients with amyotrophic lateral sclerosis (ALS), high-dose TRH temporarily provides moderate to marked improvement of functions otherwise impaired by deficiency of lower motor neurons (eg. weakness) or upper motor neurons (eg. spasticity) (Engel, Lancet 2, 73, 1983). These rapid, neurotransmitter-like effects of the hormone might be based on its binding to CNS receptors of some type.

To identify compounds with therapeutic potential equal or superior to TRH itself, we examined the binding of some TRH analogs and other compounds to "TRH receptors" (defined by high-affinity binding of [^{3}H] MeTRH) from rat brain and spinal cord. In crude membrane suspensions of pyriform cortex/amygdala, brain regions rich in an apparently single class of saturable receptors with high [^{3}H] MeTRH binding affinity (mean Kd±sem=2.0± 0.3 nM; (8), relative binding affinities (RBAs; Bouton and Raynaud, J. Steroid Biochem 9, 9, 1978) of this receptor were: TRH=100; TRH-OH =0.1; cyclo (His-Pro) (CHP), no competition at 65 µM (10,000-fold molar excess). The synthetic TRH analogs MeTRH, MK-771, RX77368, and DN1417 had RBAs of 1200, 13.3, 11.2 and 0.7 respectively. The low value for DN1417 is surprising since this analog is reported to be as potent as TRH in triggering ventral motor neuron depolarization in spinally transected rats (Ono and Fukuda, Neuropharm 21, 739, 1982) and more potent than TRH in neurobehavioral tests (Miyamoto, Life Sci 28, 861, 1981). Rat spinal cord TRH receptors had similar ligand specificities, including weak affinity for DN1417 (RBAs for spinal cord receptor were: MeTRH=1240; TRH=100; DN1417=0.8; CHP, no competition). These results discounted the possibility that the potent ventral motor neuron depolarizing effects of DN1417 (Ono and Fukuda) resulted from high affinity of spinal TRH receptors for this analog.

Cyproheptadine, an antagonist of 5-HT binding to CNS 5-HT receptors, reportedly abolishes TRH-evoked ventral motor neuron activity (Emson, Cell Surface Receptors, p 114, Wiley, 1983). However, in our in vitro competition studies, micromolar concentrations of cyproheptadine did not affect [^{3}H] MeTRH binding to brain or spinal cord TRH receptors. Together, our findings suggest that TRH and DN1417 may exert their depolarizing effects on ventral motor neurons at a site other than the TRH receptor (eg. via modulation of 5-HT receptor activity). Thus, TRH analogs that exhibit pharmacological actions on the CNS but bind poorly to the CNS TRH receptor should not be excluded from consideration as potentially therapeutic for treating ALS and other motor neuron diseases. (Supported by USC BRSG Grant 2 S07 RR05356-22).

262.9 GENETIC CONTROL OF SEROTONIN (5-HT) UPTAKE BUT NOT OF IMIPRAMINE BINDING IN BLOOD PLATELETS OF NORMAL TWINS. R. C. Arora and H. Y. Meltzer. Illinois State Psychiatric Institute, 1601 West Taylor Street, Chicago, Illinois 60612 and Dept. Psychiatry, Pritzker School of Medicine University of Chicago, Chicago, Ill. 60637.

The number of serotonin (5-HT) uptake (Vmax), and imipramine binding (IB, Bmax) sites in the blood platelets of depressed patients has been reported to be significantly lower than those of normal controls. Vmax of platelet 5-HT uptake and Bmax of IB have been proposed to be biological markers for depression. However, there is a controversey whether decreased IB in depressed patients remains constant between illness and recovery. IB sites are also believed to be a part of 5-HT uptake macromolecular complex and an allosteric modulator of 5-HT uptake. We studied 5-HT uptake and IB in the blood platelets of seven monozygotic (MZ) and eight dizygotic (DZ) twins in order to determine the genetic contribution to the Km and Vmax of 5-HT uptake and Kd and Bmax of IB.

Blood was drawn in plastic syringes and transferred to plastic tubes containing citrate-phosphate-dextrose as anticoagulant (15% V/V). Platelet-rich-plasma (PRP) was obtained by centrifugation and 5-HT uptake and IB was determined as described earlier (Arora and Meltzer, Clin. Chim. Acta., 112:225 1981., Biol. Psychiat., 19:257 1984).

Mean intrapair differences and variances in Km and Vmax of 5-HT uptake and Bmax of IB were significantly less in MZ twins than those of DZ twins, whereas no difference in the intrapair difference in Kd of IB was observed between MZ and DZ twins. There was a significant intraclass correlation coefficient (ICC) for Km and Vmax of 5-HT uptake in the blood platelets of MZ and DZ twins whereas no significant ICC was observed for Kd and Bmax of IB. These results suggest that 5-HT uptake is a stable and heritable property of platelet membranes while IB sites in blood platelets is not likely to be under significant genetic control. Thus, low platelet 5-HT uptake in depressed patient could be genetically determined. (Supported by USPHS MH 29,206, MH 30,938, and Dept. of Mental Health, State of Illinois).

262.10 REGIONAL DIFFERENCES IN CEREBELLAR SEROTONIN CONTENT IN THIAMINE DEFICIENT RATS. H. K. Strahlendorf, and J. C. Strahlendorf. Medical and Surgical Neurology and Physiology, Texas Tech Univ. Hlth. Sci. Ctr., Sch. of Med., Lubbock, Tx. 79430.

Wernicke's encephalopathy is a neurologic disorder in which patients display motor abnormalities presumably as a result of thiamine (Vitamin B_1) deficiency (TD). A relatively selective impairment of cerebellar serotonergic uptake processes as well as increased levels of 5-hydroxyindoleacetic acid (5-HIAA) in several brain regions has been demonstrated in TD. Additional studies have revealed a markedly increased turnover of serotonin (5-HT) in whole cerebellum, hypothalamus and hippocampus (see Plaitakis, et al, 1981 and 1982). With regard to the cerebellum, histopathologic changes are known to occur primarily in the anterior vermal region in man and animals with TD. The present experiments were performed to determine whether regional differences in 5-hydoxytryptophan (5-HTP), 5-HT or 5-HIAA content of the cerebellum occur in response to TD. For comparative purposes we also assayed the hippocampus (HIPP) and hypothalamus (HYPO). Male rats were fed a TD diet for 6 to 9 weeks at which time they were neurologically impaired. Controls were pair fed the identical diet with thiamine replenishment. Animals were decapitated, the brains were rapidly dissected over dry ice and each region was frozen immediately in liquid nitrogen. The cerebellum was trisected into the following regions: lateral hemispheres (CBL-L), anterior vermis (CBL-A) and posterior vermis (CBL-P). Indole content was determined by high pressure liquid chromatography with electrochemical detection. Levels from 7 to 10 animals were averaged for each region and compared to similar averages from controls. 5-HTP levels were not different in any cerebellar region. Levels of 5-HT were significantly elevated in CBL-A (99%) and CBL-P (86%). No differences were found in 5-HT levels in CBL-L. Concentrations of 5-HIAA were significantly higher in all cerebellar regions: CBC-L, 66%; CBL-P, 68% and CBL-A, 121%. In HIPP 5-HT (89%) and 5-HIAA (25%) were significantly higher. No differences were observed in levels of three indoles from the HYPO. These results suggest TD produces a relatively selective effect on serotonin metabolism in the vermal regions of the cerebellum and in the hippocampus. (Supported by the Inst. for Nutritional Sciences, TTU.)

262.11 CHANGES IN CEREBELLAR SEROTONIN RECEPTOR BINDING CHARACTERISTICS IN THIAMINE DEFICIENCY. J.C. Strahlendorf, K. E. Light+ and H. K. Strahlendorf. Phys. and Med. and Surg. Neurol., Texas Tech Univ. Hlth. Sci. Ctr., Sch. of Med., Lubbock, Tx. 79430 and +Coll. of Pharm., Univ. of Arkansas for Med. Sci., Little Rock, AK 72201

Thiamine deficiency (TD) decreases the high affinity uptake system for serotonin (5-HT) in rat cerebellar synaptosomes and alters cerebellar 5-HT metabolism (Plaitakis et al, 1982 and Strahlendorf et al, this volume). We previously showed that Purkinje cells from TD rats show qualitative and quantitative changes to iontophoretically applied 5-HT. These effects were manifest as a shift from mixed responses of excitation and inhibition to solely inhibitory actions that were elicited by significantly lower amounts of 5-HT (supersensitivity) (Lee, et al, Brain Res., in press). To further characterize these receptor changes in TD we have investigated the binding characteristics of cerebellar 5-HT receptors from control and TD rats. For comparisons of regional selectivity similar assays were performed on neocortex from the same animals. Male rats were fed a thiamine free diet and received daily subcutaneous injections of pyrithiamine 0.5 mg/kg. Controls were either pair-fed the identical diet with thiamine replenishment or were fed standard rat chow. After 15 days the TD group displayed severe neurological impairment. Rats were perfused with 70 ml of ice cold saline under pentobarbital anesthesia. Brains were dissected over dry ice and frozen in liquid nitrogen. Aliquots of tissue homogenates were incubated with 0.75-50 nM ^{3}H-d-LSD for 15 min. at 37°C. Non-specific binding was determined by incubation with cold d-LSD or by mathematical subtraction. Data was analyzed using the Ligand program (Munson and Rodbard, 1980). In cortex no significant differences were found between groups. Kd: Control = 8.2 $\pm$ 1.6 nM; TD = 4.4 $\pm$ 0.5 nM. Bmax: Control = 176.6 $\pm$ 29.7 nM; TD = 173.3 $\pm$ 26.4 nM. In cerebellum a significant decrease in receptor affinity and a significant increase in number was evident. Kd: Control = 5.9 $\pm$ 1.4 nM; TD = 13.4 $\pm$ 2.8 nM. Bmax: Control = 41.1 $\pm$ 10.4 nM; TD = 142.0 $\pm$ 54.2 nM. These data provide preliminary support for previous electrophysiologic findings of enhanced sensitivity of cerebellar neurons to iontophoretically applied serotonin. (Supported by the Institute for Nutritional Sciences, TTU).

262.12 CALCIUM-BINDING PROTEIN IN MOUSE RETINA: BIOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDIES. D.B. Farber and K.G. Baimbridge. Jules Stein Eye Inst., UCLA, Los Angeles, CA 90024 and Dept. of Physiology, UBC, Vancouver, Canada.

Calcium-binding protein (CaBP) is a 28,000 MW protein that has been found in various tissues of several species including a distinctive neuronal localization in CNS. In this latter respect, it differs from calmodulin but it shares many of the physiochemical properties of both calmodulin and other members of the calcium-binding protein family being highly acidic (pI = 4.1) and capable of binding Ca^{++} in the micromolar range. We have studied CaBP concentration and effect on phosphodiesterase activity in developing retinas of normal and degenerative (rdle) mouse littermates and its localization within the retina. Although from 5 to 12 postnatal days concentrations of CaBP were lower in rdle than in controls, they increased in both retinas following the same pattern until day 14. Thereafter, CaBP continued to accumulate in the control whereas it decreased in rdle retina. Addition of CaBP + Ca^{++} did not activate cGMP-phosphodiesterase from purified rod outer segments or the enzyme of adult rdle retina; however, CaBP + Ca^{++} stimulated the activity of 10 days normal and rdle retinas, as well as that of the adult control, 3 to 4-fold above the basal level. An affinity chromatography-purified antibody raised in rabbit against pure CaBP was used for the immunocytochemistry in normal retina. Light microscopy revealed intense staining in horizontal cell bodies and dendrites and in some amacrine cells. No staining was observed in any region of the photoreceptors. Our results indicate that in rdle retina CaBP levels are reduced and activation of phosphodiesterase is lost following the degeneration of the photoreceptors and the loss of photoreceptor-horizontal cell synaptic contact. In addition, CaBP is restricted to cells of the inner retina. These data suggest that CaBP may play a role in the modulation of Ca^{++} concentration at the retinal outer plexiform layer level. Supported by NIH grant EY 02651 (DBF) and by the Canadian MRC.

262.13 NEUROPATHOLOGY OF THE SOMATOSTATIN SYSTEM IN NEOCORTEX OF PATIENTS WITH SENILE DEMENTIA OF THE ALZHEIMER'S TYPE (SDAT). J.H. Morrison, J.B. Rogers*, S. Scherr, R. Benoit and U. DeGirolami*. Scripps Clinic and Res. Fdn., La Jolla, CA 92037 and *Univ. of Mass. Med. Ctr., Worchester, MA 01605.

There are three major prosomatostatin derived peptides present in mammalian neocortex: somatostatin-14 (SS14), somatostatin-28 (SS28), and somatostatin-28 (1-12) (SS-12) (Benoit et al., 1982). Using antibodies that have been characterized as to their specificity for these three peptides, we have initiated a detailed analysis of the immunochemical (biochemical and histochemical) distribution of these three peptides in rat, monkey, and human cortex. Data have been collected to determine the distribution of these peptides in normal human cortex, and the neuropathologic changes that occur in this system with SDAT. The general pattern of SS-positive cell body and terminal distribution in human cortex is similar to that previously described for rat (Morrison et al., 1983); however, the density of the supragranular SS-12 containing terminal field is generally higher in human cortex than in rat, and the human cortex possess a far greater degree of regional variation in the density of SS-12 positive fibers than does the rat. The temporal and frontal cortices have an extremely high density of SS-12 positive fibers, whereas the primary visual cortex has a relatively low density. We have observed the same regional patterns in new and old world monkey cortex. In addition, we have analyzed the somatostatin system in post-mortem cortical samples from patients with SDAT. There are three major neuropathologic changes in the somatostatin system found in SDAT cortices. First, there is a general decrease in the density of SS-12 positive terminals in the neocortex. This decrease is particularly striking in the temporal and frontal cortices. Second there are numerous SS-12-positive neuropathologic profiles which appear to be swollen, degenerating neurites with bulbous immunoreactive boutons. These presumed pathologic profiles are rarely present in non-SDAT post-mortem cortical samples. Third, in immunohistochemical material which has been counterstained with stains such as congo red or thioflavin that stain senile plaques, these pathologic SS-12 positive profiles are often seen in close association within a senile plaque or in the amyloid core of a plaque. Supported by grants from the MacArthur Foundation and Sam and Rose Stein Foundation (JM) and the Alzheimers Society (JR).

262.14 AMINO ACID SEQUENCE HOMOLOGY BETWEEN RABIES VIRUS GLYCOPROTEIN AND SNAKE VENOM NEUROTOXINS. Thomas L. Lentz, Edward Hawrot, Paul T. Wilson*, and David Speicher*. Depts. Cell Biology and Pharmacology and Protein Chemistry Laboratory, Yale University School of Medicine, New Haven, CT.

Rabies virus is an enveloped, negative strand RNA virus which is highly neurotropic. Evidence was presented previously that the acetylcholine receptor (AChR) might serve as a cellular receptor for rabies virus (Science 215:182, 1982). Venom from elapid and hydrophid snakes contains curaremimetic neurotoxins (Nt) which bind with high affinity to the AChR and block the depolarizing action of acetylcholine (ACh). The following is a comparison of the sequences of 1) rabies virus glycoprotein (Gp), CVS strain; 2) a long Nt, Ophiophagus hannah, toxin b; 3) a short Nt, Dendroaspis viridis, toxin 4.11.3; and 4), amino acids conserved or invariant among the long and short Nt.

1.	189	CDIFTNSRGKRASNG-NKTCGFVDERG	214
2.	30	CDGECSSRGKRIDLGCAATCPKVK-PG	56
3.		SD-H---RGTIIERGC--GCPKVK-RG	
4.		D RG GC CP V G	

The amino acid sequence of rabies virus Gp was aligned by manual comparison with the Karlsson homology alignment of long and short Nt and independently, using computer modeling (program ALIGN). The greatest degree of homology was observed between residues 189-214 of the Gp and positions 30-56 of the Nt. The Gp and long Nt are identical at 13 of 26 residues in this region. In addition to these highly homologous segments, weaker but significant homology occurs between a wider region of the rabies Gp and the entire long Nt sequence. Most significantly, the Gp shows the greatest identity with the amino acids that are highly conserved or invariant among all of the Nt and thus thought to be important for toxicity. The guanidinium group of the invariant Arg-37 is the only cationic group common to the Nt and may represent the counterpart of the quaternary ammonium group of ACh and bind to the ACh binding site on the AChR (Hndbk. Exp. Pharm., 52:159, 1979). Since the region of the Nt that interacts with the ACh site on the AChR is highly homologous to a segment of the Gp, this segment of the Gp may function as a recognition site for the AChR. The homologous portion of the Gp is most likely exposed on the surface of the protein since a probable site for N-linked glycosylation occurs at position 204. Direct binding of the viral Gp to AChR involving the indicated homologous region may contribute to the neurotropism of this virus. (Supported by NSF BNS-8203825).

262.15 IMPAIRED NEUROTRANSMITTER MODULATION OF BRAINSTEM AUDITORY EVOKED RESPONSES (BAER) IN QUAKING (qk) MICE. S.N. Shah*, A. Amochaev*, C. McCurry* and A. Salamy. Brain-Behavior Research Center, Univ. of Calif.,S.F., Sonoma Dev. Ctr., Eldridge, CA 95431.

Studies from this laboratory have provided evidence for the presence of serotonergic and cholinergic mechanism in the modulation of BAER. Alterations of receptor binding for neurotransmitters occurs in the Quaking (qk) mouse, a neurological mutant characterized by a markedly deficient myelination. In the present study we therefore investigated the effects of 5-hydroxytryptophan (5HTP), a serotonin precursor, and of nicotine and eserine, cholinergic drugs, on modulation of BAER in qk mice and their normal littermates. BAER from mice anaesthetized with pentabarbital (75mg/kg) were recorded using auditory stimuli consisting of brief clicks (20μsec duration) delivered at the rate of 23/sec at an intensity of 60dB above experimenter's threshold. The mice were placed in an electrically shielded box (8X13X13 inches) and a speaker was positioned 10 inches directly above the head. Four hundred responses were summated using a waveform eductor (Model TPM-90) or Synap I (Model 2). A baseline recording of BAER was obtained between 15 and 25 minutes after the administration of anaesthetic. Approximately 0.2ml solution of either 5HTP (75mg/kg), nicotine (100μg/kg) or eserine (20μg/kg) were injected into the shoulder muscle immediately after the baseline recording without disturbing the position of the animal. BAER recordings were then taken after 20, 40 and 60 minutes. Body temperature was maintained at 35±1.0°. Amplitudes were measured from peak to trough.

Results of our study showed that 1) administration of 5HTP to normal mice increased the amplitude of BAER peaks I, II and III, while in qk mice, 5HTP had no effect; 2) administration of nicotine to normal mice also increased the amplitude of BAER peaks I, II and III, but in qk mice nicotine increased the amplitude of peaks I and II and not III; and 3) administration of eserine to normal mice increased the amplitude of BAER peak III, but in qk mice eserine had no effect. These data indicate that the modulation of BAER by serotonergic and cholinergic mechanisms is impaired in qk mice. We suggest that this occurs as a result of, either alterations for receptor binding sites for, or altered metabolism of, these neurotransmitters in qk mice.

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262.16 N-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE BINDS TO DOPAMINE RECEPTORS IN RAT AND HUMAN STRIATE. T. Denton* and B.D. Howard. (SPON: M. Philippart). Depts. of Pathol. and Biol. Chem. Sch. of Med., Univ. of California, Los Angeles, CA 90024.

N-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (NMPTP) causes parkinsonism in primates, but not in cats or rodents (PNAS 80; 4546, 1983). Treatment over a period of several days yields an excellent model of chronic idiopathic Parkinson's Disease. An acute neuroleptic-like effect in inducing acute parkinsonism has also been noted in primates. We found that NMPTP competitively inhibits dopamine uptake into PC12 cells with an IC_{50} of 2.5 μM (BBRC 119; 1186, 1984). Because this inhibition would not account for the acute effects of NMPTP, we have examined the interaction of NMPTP with dopamine receptors in rat and human striate. Striate cortex obtained from a pool of fresh rat brains or a normal human brain, less than 25 hours old, were homogenized and centrifuged to produce a membrane suspension. The ability of NMPTP to displace [^{3}H]haloperidol binding was then measured in a standard filter binding assay. NMPTP was found to compete with 1 nM [^{3}H]haloperidol at an IC_{50} of 2.5 μM with rat brain membranes and 3.5 μM with human brain membranes. This shows that NMPTP has a broad affinity for dopamine receptors and could explain the acute neuroleptic effects of NMPTP in inducing parkinsonism. It does not explain the specificity NMPTP exhibits for primates, in vivo, nor is it clear what the relationship of this effect is to the chronic results obtained in living animals.

Supported by NIH grant MH38633.

CYCLIC NUCLEOTIDES I

263.1 ANTISERA TO A PEPTIDE FRAGMENT FROM A BAG CELL PHOSPHOPROTEIN SPECIFICALLY STAIN BAG CELL NEURONS. E.Azhderian*, S.A.DeRiemer, J.Casnellie*, P.Greengard and L.K.Kaczmarek (SPON:R.W.Aldrich).Yale Univ.,CT06510,Univ. Rochester, NY14642 and Rockefeller Univ.,NY10021.

The secretion of several neuroactive peptides from the bag cell neurons of Aplysia occurs during a long lasting afterdischarge which can be triggered in vitro by brief electrical stimulation of the pleuroabdominal connective nerve. The afterdischarge has been shown to be associated with increases in the phosphorylation state of at least two proteins. One of these phosphoproteins (BC2) is highly enriched in the bag cell neurons and has an apparent molecular weight of 21,000. The protein band that corresponds to this phosphoprotein has been eluted from SDS gels and subjected to N-terminal sequence analysis, providing a partial sequence over the first 23 amino acids (Jennings et al. J.Neurosci.,2:158,1982). In order to carry out studies on the localization and function of this phosphoprotein we have prepared antisera to a synthetic peptide sequence (Tyr-Gly-Val-His-Gly-Lys-Asn-Phe-Ala). Residues 3 - 9 of this nonapeptide correspond to residues 3 - 9 in BC2. Rabbits were immunized with the nonapeptide conjugated to thyroglobulin. We have confirmed that the antisera show immunoreactivity to the synthetic peptide and have carried out immunohistochemical staining of whole mounts of the abdominal ganglion of Aplysia. Using immunofluorescence, we have been able to detect staining above background only in the clusters of bag cell neurons. Our data indicate that, within the abdominal ganglion, this sequence is specific to the bag cell neurons. The sequence to which the antisera were prepared is also present in the precursor to the neuroactive peptides secreted by the bag cell neurons (Scheller et al. Cell 32:7,1983) suggesting that the phosphoprotein BC2 is also derived from this precursor. Experiments are in progress to determine if the bag cell peptide (ELH) precursor or any of its cleavage products are recognized by the antisera and to determine if the antisera recognize BG2.

263.2 MICROINJECTION OF PROTEIN KINASE INHIBITOR PREVENTS ENHANCEMENT OF ACTION POTENTIALS IN PEPTIDERGIC NEURONS OF APLYSIA. L.K.Kaczmarek, A.C.Nairn and P.Greengard. Depts. of Pharmacol. and Physiol., Yale Univ. Sch. of Med., New Haven, CT 06510 and Lab. Mol. Cell. Neurosci., Rockefeller Univ.,NY,NY 10021.

The stimulation of an afterdischarge in the bag cell neurons of Aplysia results in an increase in the height and width of the action potentials of these cells and is closely linked to an increase in intracellular cyclic AMP levels and to the phosphorylation of substrate proteins. Experiments in cell culture have shown that injection of the catalytic subunit of cyclic AMP dependent protein kinase enhances bag cell action potentials (Kaczmarek et al PNAS,77:7487,1980). We have now investigated the electrophysiological effects of intracellular microinjection of the protein kinase inhibitor protein (PKI) which binds to, and inhibits, endogenous catalytic subunit. Isolated cells, maintained in culture were pressure injected using microelectrodes filled at the tip with either 3.6 mg/ml PKI in carrier medium (0.6M KCl-1mM Tris pH7.8) or with carrier medium alone. Cells were stimulated by applying a series of suprathreshold depolarizing current pulses at a frequency of 1 Hz. After elevation of cyclic AMP levels with 50μM forskolin-1mM theophylline, control cells responded with an enhancement of height and width of their action potentials (mean height increase for 1st action potential 121±31% n=7) and an increase in input resistance. PKI injected cells, however, did not demonstrate spike enhancement (mean height change -17±14% n=5) nor respond with increased input resistance. PKI injection could also restore action potential height and width towards control values if injection was carried out after elevation of cyclic AMP levels.

The effects of PKI injection on the increase in action potential height and width that occurs after the onset of an electrically stimulated afterdischarge in intact clusters of cells was also investigated. The mean % increases in height and width in control cells during the first two minutes of afterdischarge were 16±7 and 31±5 (n=7) respectively. Preinjection of PKI was found to significantly attenuate this enhancement , the % changes in height and width being 3±3 and 7±3 (n=4) respectively. Our data support the hypothesis that enhancement of bag cell neuronal action potentials is mediated by cyclic AMP-dependent protein phosphorylation.

263.3 ANALYSIS OF ADENYLATE CYCLASE IN EXTRACTS OF **APLYSIA** NERVOUS TISSUE. A. Stapleton*, T. Saitoh, and J.H. Schwartz. Howard Hughes Medical Institute and the Center for Neurobiology and Behavior, Columbia University College of Physicians and Surgeons, New York, NY 10032.

Previous studies in identified Aplysia sensory neurons suggest that a persistent activation of adenylate cyclase produces the presynaptic facilitation which underlies short-term sensitization of the gill withdrawal reflex. We have used the method of Salomon to assay cyclase activity in the membrane-cytoskeleton fraction of nervous tissue, prepared in a buffer containing 2 M glycerol and 0.2% saponin.

Basal cyclase activity, each point measured in duplicate at 25°C, was 24 ± 2 pmoles cAMP/min/mg protein (N=5 independent experiments; SEM), an activity comparable to that measured in crude membranes from vertebrate brain. Serotonin, at 50 uM, stimulated the synthesis of cAMP by only 60%: as in other systems, linkage to the neurotransmitter receptor appears not to be fully maintained after extraction.

Because neurophysiological experiments suggested that the persistent activation of cyclase in short-term sensitization is mediated by a G protein (Castellucci et al. J. Neurosci. 2: 1673, 1983), we sought direct biochemical evidence for this transducer in Aplysia. Cyclase in the extract is stimulated by cholera toxin in a dose-dependent manner, with a maximum of 3.4 ± 0.3 fold basal (N = 3) at a toxin concentration of 0.4 mg/ml. One major 55 Kd protein was ^{32}P-ADP-ribosylated under these conditions, and is a candidate for the G protein. GTP-gamma-S also caused dose-dependent stimulation and was maximal (9 ± 2 fold, N=3) at 0.1 mM. Ca^{2+}/calmodulin inhibited basal cyclase activity, but the inhibition was not observed in the presence of 10 uM GTP.

The Aplysia transducer seems to be a peripheral protein, while the catalytic subunit appears to be intrinsic. In the membrane-cytoskeleton fraction washed with a low-salt buffer to destabilize the cytoskeleton, 40% of the stimulation with GTP-gamma-S was lost, while stimulation by 20 mM Mn^{2+} -- a measure of catalytic subunit activity -- was unchanged (5.0 ± 2 fold, N=5).

We are now comparing the adenylate cyclase activity in sensory cells with that in other types of neurons, and have preliminary evidence for heterogeneity of activity.

263.4 cAMP-BINDING SITES ARE OCCUPIED IN Aplysia SENSORY NEURONS DURING SENSITIZATION. S. Greenberg*, L. Bernier, E. Shapiro, J.H. Schwartz. Howard Hughes Medical Institute and the Center for Neurobiol. & Behavior, Columbia Univ. Col. Phys. & Surgeons, New York, NY 10032.

Considerable evidence indicates that short-term sensitization of the gill-withdrawal reflex in Aplysia is mediated by cAMP with the resultant activation of protein kinase. Sensitizing treatments cause an increase in cAMP in identified sensory neurons, with a time course that parallels short-term presynaptic facilitation, the electrophysiological correlate of sensitization.

Aplysia nervous tissue contains a characteristic set of 5 cAMP-binding proteins that are labeled specifically and covalently by the photoaffinity reagent 8-N_3(^{32}P)cAMP. Biochemical studies have provided evidence that most if not all of these proteins are regulatory subunits of the cAMP-dependent protein kinase.

Do these proteins actually bind cAMP during sensitization? Isolated abdominal ganglia with nerves attached were either exposed to 0.2 mM serotonin or stimulated mechanically through the left connective; both treatments have been shown to stimulate production of cAMP in sensory cells and to produce presynaptic facilitation. Five min after serotonin treatment or 30 sec after connective stimulation, sensory cells were isolated, extracted and aliquots incubated with 60 nM 8-N_3(^{32}P)cAMP either at 4°C--where endogenous cAMP remains bound to the proteins--or at 22°C, where bound cAMP is completely released. Inhibition of 8-N_3(^{32}P)cAMP labeling at 4°C relative to 22°C can therefore be taken as a measure of the extent of occupancy of each protein by endogenous cAMP.

We found that serotonin and mechanical stimulation both significantly increase occupancy of the cAMP binding proteins, indicating that the cAMP produced in sensory cells during sensitization is bound to these putative kinase regulatory subunits in vivo.

Each of the cAMP-binding proteins has a characteristic subcellular distribution. Does the compartmentalization of these presumed kinase subunits reflect functional specialization? Do particular kinase subunits mediate specific subsets of the many processes regulated by cAMP? To examine these questions, we are using a variety of cAMP-stimulating conditions to determine whether specific binding proteins are preferentially occupied.

263.5 **MEASUREMENT OF CYCLIC AMP CONCENTRATION IN *APLYSIA* NERVE CELL BODIES.** P. Hockberger and T. Yamane.* *Dept. Molecular Biophysics, AT&T Bell Laboratories, Murray Hill, NJ 07974.*

Several investigators have measured basal as well as stimulated levels of cyclic AMP in giant *Aplysia* neurons (Cedar & Schwartz, *J. Gen. Physiol., 60:* 570, 1972; Levitan & Norman, *Brain Res., 187:* 415, 1980; Bernier, Castellucci, Kandel & Schwartz, *J. Neurosci., 2:* 1682, 1982). However in each case the reported values were expressed as total cyclic AMP content per cell. Since the cell bodies are not uniform in size or shape, we have measured cyclic AMP levels normalized to both cell body volume and protein content. Using the single cell isolation procedures of Bernier et al., we have analyzed *Aplysia* cells R_2, LP1, and L_2-L_6 cluster, as well as whole ganglia. Cell body volumes were estimated assuming oblate spheroid shapes and using an average cell diameter computed by measuring the major and minor axes of each cell body. Protein content was determined using a fluorescamine method (Roche Diagnostics) as well as Lowry determinations. Cyclic AMP levels were measured using either a protein-binding radioassay (Amersham) or radioimmunoassay (New England Nuclear).

Table 1. Mean values (±SEM) of cyclic AMP concentrations in isolated *Aplysia* cells and desheathed abdominal ganglia (minus bag cells and connectives).

COMPONENT	μM	p moles/mg. protein
Cell R_2 (n=9)	16.3 ± 3.8	460 ± 87
LP1 (n=10)	17.9 ± 3.0	424 ± 126
L_2 - L_6 (n=8)	25.8 ± 4.8	468 ± 111
Abd. ganglia (n=4)	XX	16.4 ± 5.7 (S.D.)

Our preliminary results are shown in Table 1. We have not attempted to distinguish between nuclear and cytoplasmic content of cyclic AMP. Also, the glial coat which surrounds even isolated cells contributes additional uncertainty to our measurements. Nevertheless, our values of total cyclic AMP content/mg. protein for *Aplysia* abdominal ganglia are similar to those reported by Treistman & Levitan (*Nature 261:* 62, 1976). Our range of values for total cyclic AMP content per cell (not shown) is also similar to those found by both Cedar & Schwartz (1972) and Levitan & Norman (1980). In fact if one estimates the cellular diameters to have been 400-500 μm in the cells R_2 and LP1 that they examined, then basal cyclic AMP concentrations were 5-10 μM per cell in those studies similar to the values we report here.

The cellular concentration of cyclic AMP in *Aplysia* neurons appears to be similar to cyclic GMP levels in isolated rod outer segments (Woodruff, Bownds, Green, Morrisey & Shedlovsky, *J. Gen. Physiol. 69:* 667, 1977) where levels reach 40-80 μM in the dark-adapted retina. In both *Aplysia* neurons (Connor & Hockberger, *J. Physiol.* in press) and photoreceptors (Miller & Nicol, *Nature 280:* 64, 1979) fluctuations in the resting level of as little as 30 μM cyclic nucleotide results in membrane depolarization of several millivolts. The results presented here suggest that, as in photoreceptors, physiological control of membrane potential in *Aplysia* cells may be regulated by enzymes with K_m's for cyclic nucleotides in the μM range.

263.6 RAPID AUTOMATED HPLC ANALYSIS OF cAMP SYNTHESIS IN BRAIN. L.L. Lin*, C.F. Saller*, and A.I. Salama, Dept. of Pharmacology, Stuart Pharmaceuticals, Div. of ICI Americas, Wilmington, DE 19897.

A rapid automated method for measuring cAMP synthesis from either radiolabelled adenine or ATP, in intact and broken cell preparations, respectively, is described. Samples are incubated with radiolabelled precusor according to minor modifications of previously published procedures and then deproteinized with 0.3 N NaOH and 0.3 N $ZnSO_4$. After centrifugation, the superanant is injected onto a 5μ 4.6 mm x 15 cm Ultrasphere ODS reverse phase HPLC column, protected with a guard column (Rainin Instruments), using an automatic sample injector (Waters Associates). Radiolabelled cAMP (^{32}P or ^{3}H) is completely resolved from other radiolabelled compounds (precursors, etc.) using 1% methanol in a 0.2 M amnonium phosphate buffer, pH 3.0 as the mobile phase. Mobile phase flow is maintained at 1 ml/min. (Model 112 HPLC pump, Beckman Instruments). The HPLC fraction containing cAMP is located by UV detection (254 nM, Model 153 detector, Beckman Instruments; connected to a printer/plotter, Model 3390A integrator, Hewlett-Packard) of added cold carrier, and is collected into scintillation vials using an automatic fraction collector which is activiated to collect only the cAMP fraction by a peak separator (ISCO). Samples are then assayed by liquid scintillation spectrometry. Because the HPLC and fraction collection procedures are completely automated, over 50 samples can be assayed in duplicate in a single day with high reliability. The utility of these procedures will be illustrated by investigations of the effects of: β-adrenergic receptor stimulation on cAMP synthesis in tissue slices prepared from rat occipital cortex, and dopamine on cAMP synthesis in striatal membrane preparations. Data on the effects of catecholamine receptor antagonists will also be presented.

263.7 MICROTUBULE DISRUPTING DRUGS ALTER ADENYLATE CYCLASE ACTIVITY IN CULTURED NEURONAL CELLS. J.C. Mitrius, R.S. Kaplan* and M.M. Rasenick, Dept. of Physiology and Biophysics, U. of Ill. College of Medicine, Chicago, IL 60680

When synaptic membranes from rat cerebral cortex are incubated with colchicine or vinblastine, adenylate cyclase activity is augmented, and this augmentation is mediated through the GTP binding protein (Ns). When membranes prepared from NG108-15 neuroblastoma x glioma hybrid cells are incubated with colchicine or vinblastine, however, a different pattern of results emerges. Adenylate cyclase activity in the presence of NaF (20mM) GppNHp (10μM) PGEi (1μM) or forskolin (10μM) is inhibited as much as 2.3x by colchicine or vinblastine. This inhibition is dose-dependent (EC_{50} = 5x10-7 for either compound) and occurs in parallel experiments where cerebral cortex membranes display colchicine induced augmentation of adenylate cyclase.

When intact NG108-15 cells are incubated for one hour with colcemid or vinblastine, the increase in cAMP accumulation promoted by PGE1 or forskolin is diminished or eliminated. This phenomenon is dose-dependent with an apparent EC_{50} of 10^7 M for either drug. Cyclic AMP accumulated in the presence of IBMX or theophylline alone is not reduced in the presence of vinblastine or colcemid.

We have previously demonstrated that the photoaffinity GTP analog P^3-(4azidoanilido)-$P^1$5'GTP (AAGTP) will covalently label the 42KDa GTP-binding protein which activates adenylate cyclase (Ns). In synaptic membranes from rat cerebral cortex, colchicine or vinblastine increase adenylate cyclase activity by augmenting the coupling of Ns to the catalytic moiety of the enzyme. In analogy to the rod outer segment phosphodiesterase system, this is reflected by a conformational change in the 42KDa protein such that it is released from the membrane subsequent to washing. NG108-15 membranes labelled with AAGTP show a similar vinblastine-mediated release of Ns under conditions where vinblastine augments adenylate cyclase. However, under conditions where vinblastine inhibits adenylate cyclase, release of Ns is actually less (by about 50%) than in the controls. A 40KDa AAGTP labelled protein, (which may represent the inhibitory GTP-binding protein, Ni) is not released from the NG108-15 membranes subsequent to washing. A possible interpretation of these data is that colchicine or vinblastine diminish NG108-15 adenylate cyclase by constraining Ns coupling rather than facilitating coupling of Ni.

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263.8 PHOTOAFFINITY LABELLING AND CONFORMATIONAL CHANGE IN GTP-BINDING PROTEINS ASSOCIATED WITH SYNAPTIC MEMBRANE ADENYLATE CYCLASE. M.M. Rasenick and C.A. Moore*, Dept. of Physiol. and Biophys., U. of Ill. Sch. of Med., Chicago, IL 60680.

The photoaffinity GTP analog, P3-(4-azidoanilido)-P1-5' GTP (AAGTP) has been employed to convalently label synaptic membrane GTP-binding proteins which are associated with the activation or inhibition of adenylate cyclase. These proteins, with Mr of 42KDa, 40KDa and 35KDa respectively bind AAGTP optimally under different conditions. Magnesium concentrations of 5-10mM and a temperature of 30°C favors the binding of AAGTP to the 42KDa protein while at 0.5 - 1mM Mg^{++} and 23°C, the 40KDa species binds AAGTP more heavily. AAGTP binding to the 40KDa and 42KDa proteins is inversely related yet the sum of the AAGTP incorporation of the 40 and 42KDa bands is constant.

ADP-ribosylation studies are consistent with the 42KDa protein representing the GTP-binding adenylate cyclase stimulatory subunit (Ns) and the 40KDa protein representing the inhibitory subunit, (Ni). The 35KDa protein appears identical to the 35KDa protein (Ns) which has been implicated in the adenylate cyclase GTP binding protein cascade in non-neural tissues, yet in other tissues this protein has not been found to bind GTP. Identification of the 35KDa protein as Ns is based upon electrophoretic similarity to the apparently identical protein from the Rod Outer Segment. One possible interpretation of these AAGTP binding data is that the Ns and Ni compete for GTP in the activation or inhibition of adenylate cyclase.

Drugs which disrupt microtubules augment adenylate cyclase activity in membranes from neuronal tissues. Concomitant with this augmentation is a conformational change in the 43KDa protein which is reflected by the release of that species from the synaptic membrane after buffer washing. A similar phenomenon is not seen with the 40KDa or the 35KDa AAGTP-labelled proteins, which retain their association with the synaptic plasma membrane regardless of treatment and the release of 43KDa protein occurs only in neuronal tissue.

Colchicine mediated release of Ns but not Ni may be explained by the association of the former (but not the latter) with tubulin. Such an hypothesis is borne out by the coprecipitation of tubulin and Ns (but not Ns alone) with anti-tubulin antibody.

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263.9 CHARACTERIZATION OF PHOSPHODIESTERASE FROM APLYSIA PLEURAL GANGLIA. K.A. Ocorr and J.H. Byrne, Dept. of Physiol. and Cell Biol., Univ. of Texas Medical School, Houston, TX 77030

Sensory neurons involved in the defensive tail withdrawal reflex exhibit heterosynaptic facilitation which shows a temporally specific amplification in response to a classical conditioning analog (Walters and Byrne, 1983). Recently, we demonstrated that a similar classical conditioning analog applied to sensory neuron clusters from pleural ganglia results in a specific amplification of their cAMP content (Ocorr et al., 1983) that was postulated to be the result of a positive interaction of the potential facilitatory transmitter (5-HT) and Ca^{++} at the level of adenylate cyclase. Although a phosphodiesterase (PDE) inhibitor, RO 20-1724 (10^{-4}M), was included during application of the conditioning analog we could not rule out possible effects of the experimental treatment on PDE activity. As a first step in addressing this issue we have begun to characterize the PDE in Aplysia pleural ganglia.

Desheathed ganglia were pooled, homogenized, and centrifuged to provide crude membrane and cytosol fractions. PDE activity was determined by radiometric assay. Approximately 80% of the PDE activity was located in the cytosol fraction with the remaining 20% associated with the membrane. PDE activity was linear with respect to time up to 30 min. Kinetic analysis of PDE activity yielded a non-linear double-reciprocal plot indicating that there are two distinct forms of PDE in this tissue; estimated Km's for the two forms are 2.2 x 10^{-6} and 1.1 x 10^{-4}. Inhibition of PDE by RO20-1724 was dose dependent and increased with decreasing cAMP concentration. At cAMP levels corresponding to those calculated to occur in 5-HT stimulated clusters, inhibition by 10^{-4}M RO20-1724 was approximately 50%. Therefore at least part of the observed changes in cAMP levels (cited above) could be due to changes in PDE activity. PDE activity of both the cytosol and membrane fractions was enhanced by Ca^{++} and this increase was blocked by trifluoperazine, implicating calmodulin (CaM) as a mediator of this effect.

Despite the possibility that in vitro conditioning might cause a Ca^{++} activation of PDE in Aplysia neurons, we observed an increase in their cAMP levels. This would tend to support the hypothesis that the increase was the result of increased synthesis. However, we cannot exclude the possibility that decreases in activity of a second form of PDE (Ca^{++} insensitive) could contribute to enhanced cAMP levels.

263.10 REGULATION OF CALMODULIN-SENSITIVE CYCLIC GMP PHOSPHODIESTERASE BY NEUROTRANSMITTERS. T.J.Neuberger* & M.A.Ariano (SPON: W.G. Bradley). Anatomy & Neurobiology, Univ. Vermont College of Medicine, Burlington, VT 05405.

Cyclic nucleotide phosphodiesterase (PDE, EC 3.1.4.17), the enzyme which degrades the second messenger cyclic nucleotides, can be resolved into three discrete fractions from brain homogenates using anion-exchange chromatography (Biochem. 10:311, 1971). The first enzyme activity peak that elutes from the column preferentially hydrolyzes cyclic GMP, is sensitive to regulation by cytosolic calmodulin, and can be detected in the postsynaptic density organelle (Neurophar. 18:851, 1979; J. Cell Biol. 89:433, 1981; Neurosci. 10:707, 1983). The cyclic GMP PDE is the prevalent form of hydrolytic activity in brain (Brain Res. 177:301, 1979; Neurochem. Int. 5:439, 1983). We have studied the effect of various neurotransmitter compounds on cyclic GMP hydrolysis by neocortical PDE in the presence and absence of calmodulin.

PDE hydrolysis of micromolar cyclic GMP was inhibited 5-10% when assayed in the presence of 10^{-5}M met-enkephalin, dopamine, acetylcholine, or γ-aminobutyric acid (GABA). Addition of exogenous calmodulin to each of the assays containing 10^{-5}M of a neurotransmitter compound, accelerated cyclic GMP hydrolysis. The cyclic GMP PDE activity was increased 3-13% above control levels with GABA>dopamine>acetylcholine>met-enkephalin, in order of potency in the presence of exogenous calmodulin.

These data suggest that various neurotransmitters and the availability of cytosolic or non-membrane associated, calmodulin regulate PDE hydrolysis and the concommitant cellular cyclic nucleotide levels. Two transmitter systems add support for this hypothesis. 1) Some postsynaptic dopaminergic mechanisms use calmodulin. Dopamine receptor occupancy translocates membrane-calmodulin to the cytosol, enabling the protein to activate the hydrolytic PDE enzyme (Fed. Proc. 41:2273, 1982), and thereby decrease cellular cyclic nucleotide levels. 2) Postsynaptic cholinergic mechanisms however appear calmodulin-independent. Acetylcholine receptors of muscarinic subtype can be occupied and visualized *in vitro* by labeling with ^{3}H-QNB autoradiography. The receptors are clustered on cortical neurons exhibiting very robust cyclic GMP-immunoreactivity in layers 3 and 5. These investigations may elucidate a possible mechanism of interaction of calmodulin and release of neurotransmitters in control of postsynaptic cellular second messenger metabolism.

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263.11 FORSKOLIN EFFECTS ON RAT STRIATAL DOPAMINE SENSITIVE ADENYLATE CYCLASE. George Battaglia, Andrew B. Norman* and Ian Creese. Dept. Neurosci., Univ. Calif. San Diego, Sch. of Med., La Jolla, CA 92093.

In brain, dopamine (DA) can potentiate the forskolin stimulation of cAMP (Daly et al., J. Neurochem. 38:532, 1982) while in pituitary dopaminergic agonists attenuate forskolin stimulated cAMP production (Miyazaki et al., Endocrinol. 114:761, 1984). Forskolin interactions with the DA sensitive stimulatory and inhibitory components of rat striatal adenylate cyclase have yet to be detailed and are the focus of our studies. Both washed and unwashed striatal homogenates have been investigated. Our assay medium consisted of: 80mM Tris maleate (pH 7.4 at 37°C); 4mM $MgSO_4$; 0.4mM EGTA; .5mM IBMX; 5mM phosphocreatine; 50 U/ml creatine phosphokinase; .02% ascorbic acid; 100μM GTP and 1mM ATP ($2x10^6$ cpm 32P-ATP). After a 10 min preincubation of membranes with assay constituents the reaction was initiated by addition of ATP/32P-ATP and terminated after 5 min by the addition of a 2% SDS 45mM ATP solution. ^{3}H-cAMP (20,000 cpm) was added as a tracer and the separation of 32P-ATP from 32P-cAMP was accomplished by sequential elution over dowex and alumina columns. Recovery was consistently >80%.

In washed striatal homogenates there was a 62% reduction in the maximal stimulation of cAMP by DA with little difference in forskolin stimulation compared with unwashed tissue. The addition of calcium/calmodulin had little effect in restoring the response to DA or altering the forskolin stimulation. Forskolin (1μM) increased by 7 and 14 fold the Vmax of DA stimulation in unwashed and washed homogenates respectively. This stimulation was blocked by 1μM cis flupentixol. DA (100μM) significantly potentiated the forskolin stimulation of cAMP (2 fold increase in Vmax) and appeared to alter the nature of the forskolin stimulation as a function of forskolin concentration. The D-2 agonist bromocriptine could not attenuate forskolin-stimulated cAMP production. However, in the presence of 1μM forskolin, 100nM n-propylnorapomorphine (NPA) attenuated by more than 50% the stimulation by 100nM of the selective partial D-1 agonist SKF38393 (Sibley et al., Life Sci. 31:634, 1982). This attenuation was reversed by 100nM spiperone. These preliminary data indicate that in the presence of forskolin it may be possible to investigate the functional inverse coupling of DA-sensitive stimulatory and inhibitory components of adenylate cyclase in striatal homogenates. This would suggest that both systems exist on the same postsynaptic membrane fragment. Supported by PHS MH32990.

263.12 INHIBITION OF NEUROBLASTOMA ADENYLATE CYCLASE BY CANNABINOID DRUGS. A.C. Howlett* and R.M. Fleming* (SPON: M.A. Walz). Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

The properties of cannabinoids have been investigated in a variety of animal models. However, in vitro studies have failed to elucidate the mechanism of cannabinoid action at the neuronal level. We report here that cannabinoid drugs inhibit adenylate cyclase in a plasma membrane preparation from cloned neuroblastoma cells. Δ^9-Tetrahydrocannabinol (THC), as well as l-nantradol, inhibited the response to prostaglandin E1 and prostacyclin. Inhibition by cannabinoids was also apparent when secretin, vasoactive intestinal peptide, and forskolin were used as adenylate cyclase activators. The inhibition observed in these experiments was comparable in magnitude to that of carbachol at the muscarinic receptor in these membranes. The cannabinoid drugs behaved as non-competitive inhibitors at both the prostanoid and peptide receptors. The inhibition of adenylate cyclase was dose-dependent and limited to those agents that exhibit psychoactive properties. The d-isomer of nantradol was inactive, demonstrating stereospecificity of the response. These drugs may act primarily as "partial anesthetics" at the lipid bilayer of the neuronal membrane, thus perturbing receptor-cyclase interactions. Alternatively, a specific cannabinoid receptor may be postulated. Supported by NIH Grant NS16513 and a PMAF Faculty Development Award.

263.13 DOPAMINE, CYCLIC AMP, AND PROTEIN KINASE PRODUCE A SIMILAR LONG-LASTING INCREASE IN INPUT RESISTANCE IN HIPPOCAMPAL CA_1 NEURONS. V.K. Gribkoff, J.H. Ashe, W.H. Fletcher*, and M.E. Lekawa*. Department of Psychology and Division of Biomedical Sciences, University of California, Riverside, CA 92521.

Microtopical application of dopamine (DA) to CA_1 hippocampal pyramidal neurons in vitro can result in the initial depression of population postsynaptic responses. This short-lasting effect is often followed by a long-lasting enhancement of subsequent responses [Gribkoff & Ashe, Br. Res. 292 (1984):327]. Intracellular recordings reveal that DA produces a short-lasting membrane hyperpolarization (HP) accompanied by a decrease in input resistance (R_i) [Ashe & Gribkoff, submitted; Benardo & Prince, J. Neurosci. 2 (1982):415]. The initial membrane HP is frequently followed by a prolonged membrane depolarization (DP) which is accompanied by an increase in R_i [Ashe & Gribkoff, submitted].

To explore possible cellular mechanisms for these effects, 8-bromo-cyclic AMP was microtopically applied to CA_1 neurons, producing a short-lasting HP and a decrease in R_i. Following this initial HP a longer-lasting DP and increase in R_i was observed in some neurons. These effects were not observed following application of control solutions. The pattern produced by 8-bromo-cyclic AMP was very similar to that observed following DA application. Intracellular injection of cyclic AMP from the recording electrode did not produce consistent alteration of membrane potential, but did result in an enduring increase in R_i.

Intracellular injection of a highly purified fraction of bovine heart cyclic AMP-dependent protein kinase catalytic subunit (PKC) [Fletcher & Byus, J. Cell Biol. 93 (1982): 719] also produced no consistent changes in membrane potential, but an increase in R_i was noted. Injection of heat-inactivated PKC had no effect on R_i. Injection of bovine brain cyclic AMP-dependent protein kinase inhibitor protein (affinity column purified) had no significant effect on short-term effects of DA, but did reduce or eliminate DA-induced increases in R_i. These results suggest that long-lasting effects of DA on R_i of hippocampal pyramidal neurons are mediated by cyclic AMP and protein phosphorylation. Supported by NIH grant BRSG-RR07010-17 to JA.

263.14 EFFECTS OF FORSKOLIN ON cAMP LEVEL AND EXCITABILITY IN HIPPOCAMPAL SLICES. S. Lin-Liu, C. Cain*, S.M. Bawin, and W.R. Adey. Loma Linda University, University of California at Riverside and VA Medical Center, Loma Linda, CA 92357.

Forskolin, a potent activator of adenylate cyclase, mimics the action of cAMP in brain tissue (Madison, D.V. and Nicoll, R.A., Nature 299:636, 1982.). This effect is presumably due to an increase in intracellular cAMP levels. However, a direct correlation has not yet been made between forskolin-induced changes in the level of cAMP and tissue excitability. The present experiments coordinated these biochemical and electrophysiological aspects in the hippocampal slice preparation. Transverse hippocampal slices (400 μm) were obtained from male Sprague-Dawley rats. For biochemical studies, the slices were incubated at 33-35°C in Kreb's saline saturated with 95% O_2 - 5% CO_2. Warmed gas was also circulated continuously above the slices. Forskolin (1-100 μM) was added after 35 min of preincubation. Phosphodiesterase activity was terminated by rapid microwave heating at the end of incubation (5-30 min). The tissue was then kept frozen overnight in sodium acetate (0.05 M, pH 4). After thawing, the cAMP content of the supernatant was determined by radioimmunoassay. Preliminary data showed that forskolin-stimulated cAMP accumulation occurred within 5 min and reached a maximum level in 10-20 min in a dose-dependent fashion. Maximal (from basal cAMP level of 5 to about 200 pmole/mg protein) and half maximal stimulation appeared at 50 and 10 μM forskolin. Increases in cAMP level were detectable at 1 μM. Following stimulation by 50 μM forskolin, cAMP level returned to base-line after 10 min of incubation in control medium. The slices used for electrophysiological studies were constantly perfused at 33-35°C with O_2 - CO_2 saturated Kreb's saline. Population spikes evoked by test-pulse stimulation in stratum radiatum were recorded in the CA1 cell layer. After base-line recording of minimum 10 min, the control solution was switched to forskolin-containing Kreb's saline. Preliminary data showed that the amplitude of the population spike was increased (50% or more) after 10-20 min of perfusion with 50 μM forskolin. This potentiation was reversed by perfusion for 10-30 min with control solution. These results followed closely the responses in cAMP accumulation and suggested a causal relationship between the two effects.

(Supported by the Department of Energy and Southern California Edison Company.)

263.15 FORSKOLIN DECREASES A VOLTAGE-DEPENDENT OUTWARD CURRENT IN EMBRYONIC CHICK DORSAL ROOT GANGLION (DRG) NEURONS. Kathleen Dunlap. Physiology Dept., Tufts Med Sch., Boston, MA 02111.

The duration of the action potential recorded from the soma membrane of embryonic chick DRG neurons is controlled by a balance of inward Ca current and outward K current. Forskolin, an adenylate cyclase activator, reversibly increased action potential duration (APD) in a dose-dependent manner, with an ED_{50} of 15 μM. This effect was accompanied by an increase in input resistance, a small (2-3 mV) depolarization of the resting potential, and a decrease in the negative afterpotential following spike repolarization, Fig.A.

The voltage-dependent outward current measured under voltage clamp (in a solution containing 10mM Co and 0.1μg/ml TTX) was decreased by forskolin (Fig.B). The activation kinetics appeared unaffected, but the sag in the current (inactivation) was accentuated by the drug.

This effect of forskolin most likely results from an increase in adenylate cyclase activity since 1) cholera toxin (10μg/ml), another cyclase activator, also increased APD and 2) 2',5'-dideoxyadenosine, an inhibitor of forskolin-activated increases in cyclase activity in other preparations blocked forskolin's effects on APD.

The increase in APD produced by forskolin is in marked contrast to the norepinephrine (NE) and GABA induced decrease in APD (via a decreased Ca current) previously reported for these cells. Forskolin concentrations which produced maximal effects on APD (25μM) did not inhibit the ability of NE to decrease APD.

These results suggest that a rise in intracellular cyclic AMP levels decreases a voltage-dependent K current in embryonic chick sensory neurons, but has no effect on the NE modulation of Ca channels in these cells.

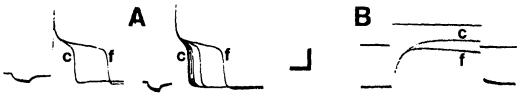

Figure: c=control, f=forskolin. Right panel in A shows return to control following forskolin (10 sec between traces). Cal: A, 40mV,10msec; B, 40nA,50mV,10msec.

This work was supported by a Grant-In-Aid (83 856) from the American Heart Association.

263.16 EXCITATION OF AREA POSTREMA NEURONS BY INSULIN AND PEPTIDES AND THE ROLE OF CYCLIC NUCLEOTIDES D. Carpenter and D. Briggs* (SPON: J. Ramaley) NY State Dept. of Health, Albany, NY 12201.

The area postrema (AP), located at the floor of the fourth ventricle outside of the blood brain barrier, is known to function as a receptor zone for humoral agents which trigger the emetic reflex. We have studied the actions of transmitters, peptides and hormones on the activity of single units in the AP in anesthetized dogs, and have attempted to correlate single unit excitation with the ability of each substance to induce emesis. Our previous results (Cell. Mol. Neurobiol. 3:113:1983) have shown that AP neurons are silent at rest but can be excited with short latency and brief discharge by glutamate. A variety of small peptides are emetic and excite AP neurons, including the enkephalins, thyrotropin releasing hormone, gastrin, vasoactive intestinal polypeptide, angiotensin II, neurotensin, vasopressin and oxytocin. Dopamine, apomorphine, serotonin, norepinephrine and histamine are also excitatory. In contrast to glutamate these substances induce a long latency, very low frequency and long duration discharge often lasting several minutes. A second application of an excitatory substance often provoked spontaneous discharge.

Since high doses of insulin have been reported to be emetic in humans, we applied insulin by ionophoresis onto AP neurons and also tested for behavioral emesis on IV administration. Insulin excited 52% of 60 neurons examined, resulting in a prolonged discharge similar to that of other excitatory substances. Neither glucose or zinc had effects. On IV administration 50% of dogs tested showed emesis with 8 I.U. insulin IV.

Because so many substances showed a similar pattern of discharge, we suspected that the responses were mediated by a common second messenger. Consequently we applied 8-bromo-cyclic AMP, forskolin and theophylline by ionophoresis. Both 8-bromo-cyclic AMP (59% of 22 cells) and forskolin (26% of 35 cells) were excitatory. Theophylline had no effect. To test the possibility of cyclic AMP involvement behaviorly we pretreated unanesthetized dogs with theophylline (25mg/kg) 30 min prior to testing with angiotensin II and insulin, each at three concentrations. For both theophylline caused a shift of the dose response curve to the left. These results provide one of the first demonstrations that insulin can directly excite neurons and are consistent with the premise that at least insulin and angiotensin excitation of AP neurons involves activation of an adenylate cyclase.

263.17 EFFECTS OF METHYLXANTHINE DERIVATIVES ON INSECT BEHAVIOR. J.A. Nathanson. Dept. of Neurology, Harvard Med. Sch. and Neuropharmacology Research Lab., Mass. General Hospital, Boston, MA 02114.

Caffeine, theophylline, and theobromine are found in certain species of plants at relatively high concentrations, yet the possible natural function of these compounds is unknown. We report here that, at concentrations found naturally in plants, caffeine and certain other methylxanthines can alter behavior in certain, but not all, species of insects. In Manducca sexta, methylxanthines incorporated into artificial media, cause hyperactivity, tremors, decreased feeding, and stunted growth. Applied as a spray to leaves used as natural food, methylxanthines decrease the rate of leaf consumption by Manuducca. In solution, methylxanthines disrupt swimming behavior of mosquito larvae, resulting in their death. The rank order potency of various methylxanthines in disrupting insect behavior correlates well with their rank order potency as inhibitors of insect phosphodiesterase activity and poorly with their rank order potency as adenosine antagonists. In addition, when combined with certain other activators of insect adenylate cyclase, the methylxanthines act in a synergistic manner to alter behavior. Other biochemical data suggest that the mechanism of action of the methylxanthines in the observed alterations of insect behavior is through an action on tissue cyclic AMP levels and not through effects on calcium mobilization or on adenosine receptor blockade. These findings have implications for the development of pesticides and pesticide synergists.

264.1 MORPHOMETRIC ANALYSIS OF THE PYRAMIDAL TRACT OF THE RAT WITH EMPHASIS ON ITS UNMYELINATED FIBER POPULATION.

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The pyramidal tract is generally considered as a mainly myelinated fiber system. However, recent studies e.g. Reh, T., Kalil, K., J. comp. Neurol., 205: 77, 1982; Leenen, L. et al., Brain Res. 246: 297, 1982, revealed the presence of considerable numbers of unmyelinated fibers in this tract. In view of these findings we carried out a detailed E.M. morphometric analysis of the pyramidal tract of the rat at two different levels, i.e. pyramis medullae (P.M.) and the second cervical segment (C2).

At PM level a total of 140,000 ± 22,000 unmyelinated fibers and 103,000 ± 16,000 myelinated fibers was found. The diameters of unmyelinated fibers range from 0.03 to 1.21 µ with a mean of 0.17 µ, whereas myelinated fibers measured with meylinsheath range from 0.25 to 6.03 µ, with a mean of 1.19 µ. Myelinated axon profiles vary between 0.13 and 4.92µ (mean: 0.81 µ). Partitioning of the tract under study, revealed no statistical significant differences between medial/lateral, ventral/dorsal and central/peripheral regions with regard to both numbers of fibers and diameter spectra. Also no statistical significant right/left differences were elucidated.

At C2 level a total of 35,000 ± 9,000 unmyelinated fibers and 43,000 ± 7,000 myelinated fibers appeared to be present. Data concerning fiber diameter spectra, subregions and comparison right/left are similar to those found at PM level.

Comparison of the data derived from the two levels studied shows that:

(1) there is a decrease in fiber number between PM and C2 for both myelinated and unmyelinated fibers;
(2) the decrease in the number of unmyelinated fibers is much larger than that for the myelinated fibers;
(3) despite the substantial decrease in fiber number of both subpopulations, no specific diameter subset leaves the tract between the two levels studied.

We are currently performing experiments to determine the origins and sites of termination of the unmyelinated subset of the pyramidal tract.

264.2 DESCENDING CHOLINERGIC PROJECTIONS FROM THE MESOPONTINE TEGMENTUM. D.B. Rye, H. Lee*, B. Ronnett*, B.H. Wainer. Departments of Pathology and Pediatrics, The Univ. of Chicago, Chicago, IL 60637.

The presence of cholinergic projections from the reticular formation to the thalamus suggests that cholinergic neurons of the mesopontine tegmentum may be part of the ascending reticular activating system (Mesulam et al., Neuroscience 10:1185, 1983). Many of these cholinergic neurons, however, lie within the boundaries of the pedunculopontine nucleus (PPT) which has been shown to have reciprocal connections with the extrapyramidal motor system (Saper and Loewy, Brain Res. 252: 367, 1982). The present study was undertaken to better define the anatomy of PPT cholinergic neurons by first investigating their possible descending projections. Rats received injections of HRP and/or WGA-HRP into either a) the caudal pontine reticular formation (PRF); b) the medullary reticular formation (MRF); or c) the cervical and thoracic spinal cord. Sections were processed for the visualization of the retrograde cell marker alone or in combination with choline acetyltransferase (ChAT) immunocytochemistry. In each case retrogradely labeled neurons were observed predominantly ipsilaterally in the PPT. A significant number of these neurons (30-50%) also stained positively for ChAT only after injections into the PRF and MRF. Control injections in the vestibular nuclei, inferior olive and nucleus tractus solitarius revealed little retrograde labeling in the PPT. This study supports the view that the PPT represents a functional link between the basal ganglia and lower motor systems, and demonstrates that cholinergic neurons contribute significantly to these descending projections. Supported by USPHS HD-04583, NS-17661, 5-T32GM07281, and the McKnight and Brain Research Fnds.

264.3 SPINAL TERMINATION OF FIBERS ARISING FROM THE PRIMARY SENSORIMOTOR CORTEX OF RATS. E.J. Casale*, A.R. Light and A. Rustioni (SPON: J. Greenspan). Depts. of Physiology and Anatomy, Univ. of North Carolina, Chapel Hill, NC 27514.

The terminal pattern of cortical fibers in the spinal cord has previously been studied using silver impregnation of degenerated fibers and autoradiography. A more sensitive and direct anatomical approach is represented by the anterograde transport of lectin-conjugated horseradish peroxidase (WGA-HRP). This tracer was recently employed for a reevaluation of the corticospinal tract (CST) in cats and monkeys. In the present experiments the same method was used to evaluate the termination pattern of CST fibers in Sprague-Dawley rats. WGA-HRP (2%, Sigma) was pressure-injected by multiple penetrations into the left primary sensorimotor cortex inclusive of area 4, as defined by the pattern of cerebral blood vessels and available somatotopic maps. Histological processing showed that the injection resulted in uniform infiltration of the intended areas without involvement of subcortical gray. After 3 days survival, rats were perfused with standard aldehyde fixatives, and 40µm thick, transverse sections were cut from cervical segments and processed with tetramethyl benzidine as histochemical substrate for the visualization of anterogradely labelled fibers.

Labelling in the spinal white matter occurred only in the ventral-most portions of the dorsal columns contralateral to the injection side. Within the gray matter terminations were moderately dense but nonuniform in laminae VII and VIII; in lamina IX, terminations seemed to be largely absent except for the occasional presence of granular reaction product. Throughout most of the dorsal horn, labelling was dense and uniform, but in the most dorsal laminae (I and II) labelling was, on the whole, very sparse. However, in a restricted medial sector of the dorsal horn at C6 through C8, dense labelling extended up to the dorsal margin of the gray matter. On the basis of cytoarchitectonic and immunocytochemical observation using substance P and leu-enkephalin as antigens, it is questionable whether this medial region of the dorsal-most part of the spinal gray can be considered as part of laminae I and II. The results suggest that either this medial labelling reflects a dense, focused, and direct corticospinal projection to laminae I and II, or the structural organization of these two laminae, in the medio-lateral extent, is not as uniform as hitherto believed. (Supported by NINCDS Grants NS16433 and NS16264.)

264.4 DENDROARCHITECTONICS OF RETICULOSPINAL NUCLEI OF THE RAT. D.B. Newman, Department of Anatomy, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.

While cytoarchitectonic and hodological methods suggest that brainstem reticulospinal nuclei (BRN) are complexly organized, previous Golgi studies claimed that BRN comprise a homogenous population with respect to neuronal morphology. To determine whether this is true, neurons of the various BRN of the rat were either backfilled with HRP from spinal injections or stained with a Golgi-Kopsch variant. At least 26 distinct BRN could be distinguished on the basis of axonal trajectories or dendroarchitectonics. Some BRN contained neurons whose dendritic arborizations are radially symmetrical (e.g., nucleus reticularis (NR) ventralis pars beta, NR gigantocellularis, nucleus raphe (NRa) magnus, NR pedunculopontinus pars compactus and pars dissipatus, NR cuneiformis, and NR subcuneiformis). Some BRN contained neurons whose dendritic arborizations exhibit a pronounced dorsomedial to ventrolateral slant (e.g., NR dorsalis, NR parvocellularis, NR pontis caudalis alpha, NR pontis caudalis beta, the A5 noradrenergic group and the NR subceruleus) while others contain neurons whose dendritic arborizations slant from dorsolateral to ventromedial (e.g., NR ventralis pars alpha, locus ceruleus).

The majority of dendrites of NR paragigantocellularis dorsalis course dorsally, those of NR pontis oralis pars medialis course medially, and those of NR pontis oralis pars lateralis course laterally. The dendrites of neurons in NRa obscurus course vertically, while those of several nuclei (e.g., NR magnocellularis pars alpha and beta, Kolliker-Fuse nucleus) course horizontally. Several BRN nuclei exhibit a variety of characteristic dendritic arborization patterns (e.g., NR paragigantocellularis lateralis, NRa pallidus, NRa dorsalis).

Rat BRN can also be distinguished on the basis of laterality and funicular trajectories of their axons. Since the various BRN are distinguishable on the basis of dendritic orientation and axonal trajectories, they may play distinct roles in brainstem modulation of spinal motor, sensory or autonomic activity. (Supported by USUHS grants C07003 and R07047).

264.5 PROJECTION NEURONS OF THE NUCLEUS TEGMENTI PEDUNCULO-PONTINUS OF THE RAT: A COMBINED GOLGI AND HRP STUDY. L. R. Lutze,* and J. A. Rafols. Depts. of Occupational Therapy and Anatomy, Sch. of Phar. and Allied Health and Sch. of Med., Wayne State Univ., Detroit, Mi. 48201.

Three morphologically distinct types of neurons can be distinguished in Golgi preparations of the nucleus - tegmenti pendunculopontinus (PPTg) of the adult rat. Neurons of the first type are found primarily in the more rostral portion of the nucleus. These neurons have large, polygonal or fusiform cell bodies (25-47 μm, max. soma dia.) with 4-5 dendritic trunks ranging in diameter from 2.5-6.3 μm. The dentrites are uniformly thick and smooth with occassional spines, dendritic appendages and axon-like processes. Neurons of a second group (20-24 μm) are found in the mid-to caudal portion of the nucleus and are characterized by multipolar somata with 3-5 dendritic trunks ranging in diameter from 1.3-6.3 μm. The dendrites are thin, radiate symmetrically and tend to be smooth with sparse spines and an occasional dendritic appendage. Neurons of a third type are also localized in the caudal part of the nucleus and are characterized by small to medium fusiform cell bodies (12-19μm) with 2-4 dendritic trunks ranging in diameter from 1.3-5.0 μm. The dendrites of these neurons are few in number and varicose with heterogeneous appendages. Some of these dendrites appear to terminate as axon-like processes.

Sterotaxically guided injections (20-30 nl; 20% Sigma Type VI HRP) were placed in the cervical spinal cord or in the corpus striatum in order to retrogradely label the neurons in the PPTg that project either to the spinal cord or to the rostral forebrain. After either spinal cord or corpus striatum injection, retrogradely labelled small and medium neurons appear localized in the mid-to caudal portion of the PPTg. The soma and dendrite morphology of the HRP labelled cells suggests that they may represent the second and third neuronal types from the Golgi preparations. Thus, it appears that the territories of the groups of neurons projecting to the spinal cord and basal ganglia overlap. In addition, the projections to the spinal cord and the corpus striatum from the PPTg neurons are distributed bilaterally with more concentration ipsilaterally. Supported in part by a neuroscience grant (306-6166) from the Neuroscience Program, Wayne State University.

264.6 COLLATERALS OF RUBROSPINAL NEURONS TO THE NUCLEUS INTERPOSITUS IN CATS,A RETROGRADE FLUORESCENT DOUBLE LABELING STUDY,WITH AN ATTEMPT TO DETERMINE THE UPTAKE ZONE OF FB AND DY. F.Condé* and H.Condé*,Laboratoire de Neurobiologie du Développement,Bat.440,Université PARIS-sud,91405-Orsay France (Sponsor: F.BAHLS,University of Washington)

The collaterals of rubrospinal neurons to the nucleus interpositus anterior of the cerebellum (NIA) were studied in cats,using the fluorescent double labeling technique. Two combinations of fluorescent tracers were used:"fast blue"(FB) with"diamidino yellow"(DY) or "nuclear yellow" (NY);FB and DY were both used either in the cerebellum or in the spinal cord,NY only in the spinal cord.

In the injection area different zones could be distinguished:the FB injection area consisted of three concentric zones (I,II and III) surrounding the needle track(zone 0), the extension of the zone I depending on the survival time, whereas the DY injection area consisted in only two concentric narrow zones (I and II) surrounding the needle track (zone 0).

Maximally 19% of the rubrospinal neurons were double-labeled from the contra-lateral NIA and rubrospinal tract, versus 37% in rat (1).The distribution of rubrocerebellar neurons (single + double labeled)seemed to depend only on the zone 0,for both DY and FB.When the zone0was not in the NIA no labeling was observed in the red nucleus,even though the zones I and II were superimposed to the NIA,especially for FB where the zone I could be very large.When the zones 0 (in cases of multiple injections) were distributed in the whole NIA,rubrocerebellar neurons were found among the whole rubrospinal neuron population.When the zones 0 were restricted to the posterior part of NIA, rubrocerebeliar neurons were found mostly in the dorsomedial area of the red nucleus.These results suggest a topography in rubro-interpositus projections and are in keeping with those of Courville and Brodal(2).The uptake zone for DY and FB seemed to be the zone 0,the area where nervous tissue is damaged.

(1):Huisman,Kuypers,Condé and Keizer,Brain Res.264(1983)181-196.

(2):Courville and Brodal,J.Comp.Neurol.126(1966)471-485.

264.7 AN AUTORADIOGRAPHIC STUDY OF ASCENDING NUCLEUS GIGANTOCELLULARIS PROJECTIONS IN THE RAT. R.P. Vertes, R. Waltzer, and G.F. Martin. Mercer University, School of Medicine, Macon, GA 31207, and Dept. of Anatomy, The Ohio State University, School of Medicine, Columbus, Ohio 43210.

Forty rats received injections of tritiated leucine at various levels of the brainstem gigantocellular tegmental field. After a post-injection survival period of 12-14 days, the rats were killed, their brains removed and processed according to previously described autoradiographic methods (Martin et al., J. Comp. Neurol. 187:373, 1979). Injections confined to the medullary nucleus gigantocellularis (NGC) yielded the following distribution of ascending projections.

The majority of ascending NGC fibers coursed bilaterally through the central part of the pontine and midbrain tegmentum giving off profuse projections in passage. A secondary but prominent ascending route was the medial longitudinal fasciculus. In the pons, two bundles of fibers exited dorsally from the main bundle, i.e., dorsolaterally to the parabrachial complex and the subcoeruleus area and dorsomedially to the dorsolateral tegmental nucleus. At the caudal midbrain the main bundle was the source of strong projections to the retrorubral (RR) nucleus (A8 area) and a region of the central gray (CG) directly above the oculomotor nucleus. At this same level a large contingent of fibers left the main bundle to establish a permanent position on the lateral border of the CG. From there fibers distributed to intermediate layers of the superior colliculus and to a distinct mid-central region of the CG lateral to the aqueduct. At the rostral midbrain, an extremely prominent termination site for NGC fibers was the anterior pretectal nucleus (APN). The entire extent of the APN was heavily labeled in all cases. At the diencephalon, NGC projections became essentially confined to a dorsal and ventral bundle in accord with classical descriptions in other species. The dorsal bundle terminated strongly in the centromedian-parafascicular complex (CM-PF) and less in each of the anterior intralaminar nuclei. The ventral bundle distributed widely over the ventromedial diencephalon heavily innervating field H_1 of Forel and zona incerta and moderately the lateral and supramammillary nuclei and medial and lateral hypothalamus.

In summary, the major upper brainstem and forebrain termination sites for NGC fibers were: specific regions of the CG, superior colliculus, parabrachial complex, RR, central pontine and midbrain tegmental fields, APN, CM-PF, field H_1 of Forel and zona incerta. Supported by NSF Grants BNS-8403544 to RPV and BNS-8309245 to GFM.

264.8 DEMONSTRATION OF ANDROGEN RECEPTORS IN CRANIAL NERVE MOTOR NUCLEI. W.H.A.Yu and M.Y.McGinnis*, Department of Anatomy, Mount Sinai School of Medicine of the City University of New York, NY, NY 10029.

Testosterone propionate (TP) accelerates axonal growth of the hypoglossal nerve following nerve lesion (Yu et al., Exp. Neurol.,77:129,1982; 80:349,1983). Because uptake of androgens into the somatic motor nuclei has been shown autoradiographically, we sought to determine the presence of androgen receptors in the hypoglossal (HGN) and facial motor (FMN) nuclei. Castrated, adult rats, 200-250 g, were used to measure both cytosolic and cell nuclear androgen receptor binding by the method of McGinnis et al. (Brain Res.,275:75,1983). Tissues from HGN and FMN were dissected out under a stereomicroscope. Tissues from combined hypothalamus, preoptic area, amygdala and septum (HPAS), shown previously to contain androgen receptors (Brain Res.,275:75,1983), were assayed simultaneously along with HGN and FMN for comparison. Samples from 6-10 rats were pooled for each assay. For cytosol assays, 4 nM ^{3}H-R1881 was used as ligand. Cytosolic androgen receptor binding was present in HGN and FMN of castrated rats which was at a level 5-6 fold higher than that of castrates receiving TP (1 mg), s.c., 1-2 hr before killing. TP treatment virtually eliminated cytosolic androgen receptor binding in HGN and FMN. Using 4 nM ^{3}H-R1881 or ^{3}H-DHT (dihydrotestosterone) as ligand, cell nuclear androgen receptor binding in HGN was assessed by the exchange method. Castrated rats received 1 mg TP, s.c., 1-2 hr prior to killing; uninjected castrates served as controls. Cell nuclear androgen receptor levels in TP-treated rats were increased over those of controls. Both ^{3}H-R1881 and ^{3}H-DHT yielded comparable results. The androgen receptor binding in cytosol and cell nuclei was specific. Displacement curves, using DHT, estradiol, progesterone or corticosterone as competitors (10^{-9}-10^{-6}M), showed that DHT inhibited both cytosolic and cell nuclear androgen receptor binding in HGN and FMN in a manner similar to that previously shown for HPAS. Corticosterone and estradiol competed for HGN and FMN androgen receptors only at the highest concentrations. We propose that androgens via their receptors, first demonstrated here in motor nuclei of rat brain, may play a role in motor neuron function and may provide trophic effects on neurons.

(Supported by an institutional grant from the Mount Sinai Medical Center)

264.9 ORGANIZATION OF THE PUDENDAL NERVE IN THE MALE AND FEMALE RAT. K.E. McKenna and I. Nadelhaft, VA Medical Center and Depts. of Pharmacology and Neurosurgery, Univ. of Pittsburgh, Pittsburgh, PA 15240.

Mature male and female Sprague Dawley rats were used in this study to compare the organization of the pudendal nerve in the two sexes. Experiments included 1) incubating the cut pudendal nerve in diamidino-2-phenylindole HCl (DAPI), Fast Blue (FB) or horseradish peroxidase (HRP) and 2) injecting individual perineal muscles with these substances. In both sexes, incubation of the motor branch of the pudendal nerve labelled two motoneuron nuclei at the L5-L6 border of the spinal cord. These nuclei are the dorsomedial (DM) and dorsolateral (DL) cell columns described by Schröder (1980). We injected the external urethral sphincter (US) and the external anal sphincter (AS) in both sexes and the ischiocavernosus (IC) and the bulbocavernosus (BC) muscles in the male only. (The IC and BC were too fine to inject in the female). In both sexes, neurons innervating the AS were located only in DM and neurons innervating the US were located only in DL. In male, when the AS and BC were injected separately with different labels, it was revealed that the motoneurons innervating these two muscles were totally intermingled in DM. In contrast, when the US and the IC were similarly labelled, the motoneurons innervating the US occupied the lateral portion of DL and the IC neurons the medial portion. However, both sets of neurons had the same rostrocaudal and dorsoventral distribution. Cell counts of the pudendal neurons indicate that DL and DM are sexually dimorphic as reported by Breedlove and colleagues. In males DL has approximately 2 times the number of DL neurons in the female and DM contains approximately 3.5 times the number of DM neurons in the female. Cell counts following muscle injections indicate that US neurons account for approximately 1/2 of the DL neurons in the male and virtually all of the DL neurons in the female. Similarly, the AS neurons comprise 1/4 to 1/3 of the DM neurons in the male and virtually all of the DM neurons in the female. The number of neurons innervating the two sphincters are comparable in males and females. We conclude that in the female, these two cell columns subserve primarily an excretory function, while in the male these nuclei are also involved in sexual function. The sexual dimorphism of these nuclei is reflected in the relatively underdeveloped nature of the IC and BC in the female.

264.10 THE INCREASE IN NUMBER OF NEURONS IN SOLEUS MOTOR NEURON POOL IS ACCOMPANIED BY A SHIFT IN LOCATION OF THE NEURONS IN THIS POOL. W. D. Martin* and R. L. Van Buskirk* (SPON: A. H. Hassen). WV School of Osteopathic Medicine, Lewisburg, WV, 24901.

A prior study (VanBuskirk and Martin, 1982) reported that the number of motor neurons and the proportion of slow oxidative fibers increased in soleus muscle through adulthood. In order to better understand this increased innervation we examined the location of soleus motor neurons over the course of maturation. Soleus was isolated and injected with 36 ul of 20% HRP in 12 male rats weighing 54 to 560 g. In 7 rats (SA) all nerves supplying muscles of the crus were left intact. In 4 rats (SD) all nerves to muscles of the crus were cut with the exception of the primary nerve to soleus passing from between gastrocnemius and plantaris muscles. In 1 rat (SN) this primary nerve to soleus was cut and all other nerves of the crus were undisturbed. After 24 to 72 h the rats were killed and soleus muscle was removed and processed for muscle fiber types (Martin and Romond, 1975). The spinal cord was reacted for HRP using TMB (Mesulam, 1978). In the SA rats the number of labeled neurons increased as the body weight and proportion of slow oxidative fibers increased. A significant increase in labeled neurons was noted in the cell column medial to the marginal columns in the ventral horn. In addition, a significant increase in the number of labeled neurons was noted in the dorsolateral marginal cell column. In the SD rats greater than 90% of the neurons were located in the central column, with little difference in number between large and small rats. In the SN rat labeled neurons were found in both the central and marginal cell columns. These results suggest that 1) soleus motor neurons are found in more than one cell column in the ventral horn; 2) soleus muscle is innervated by more than one nerve in the adult; 3) the primary nerve to soleus does not contribute significantly to the increase in number of soleus motor neurons; 4) significant increases in labeled neurons innervating soleus are found in both the central and dorsolateral cell columns as the rat grows. (Supported by WVSOM grant # 4-83).

264.11 POST-NATAL DEVELOPMENT OF DOPAMINE-BETA-HYDROXYLASE IMMUNOREACTIVE FIBERS OF RAT SPINAL CORD. R. Aramant* and L. Giron, Neurology Service, VA Med.Center, Kansas City, Mo. 64128 and Dept. of Neurology, Kansas Univ. Medical Center, Kansas City, Kansas

Development of noradrenergic fibers in the spinal cord has been previously studied by glyoxylic acid-induced histofluorescence which does not distinguish between dopaminergic and adrenergic terminals. In the present study, we used dopamine-beta-hydroxylase (DBH) immunoreactivity for sensitive, more nearly specific localization of noradrenergic fibers.

We examined the pattern of DBH-immunoreactive fibers in the cervical, thoracic, and lumbar regions of the spinal cords of Sprague-Dawley rats at 0, 6, 14, 30, and 90 days of life. At day 0: a) In the cervical cord, DBH staining was restricted to grey matter, was more dense in ventral horn (VH) than dorsal horn (DH), and was notable in the dorsal commissural nucleus. b) In the thoracic cord, the densest staining was in the intermediolateral cell columns (IMLC's) and the transverse connections between them. c)In lumbar cord, the VH especially its ventral margin was prominently stained, while the DH was very sparsely stained. By day 6, staining was dense in all regions; the same general pattern prevailed, but sparse staining was present in ventral white matter. By day 14, staining was generally much denser; but in all regions, the new finding was a pronounced staining in ventral white matter; from this age on, radial extensions of IMCL into surrounding white matter were well-developed. By day 30, DBH-immunoreactive fibers were more numerous; a fine network of fibers was most pronounced in the ventral grey; but fibers were reduced in number in the ventral white matter. At day 90, specific staining was generally denser but was again nearly confined to the grey matter. Transverse connections between the IMLC's were periodically organized.

In general,the developmental pattern of noradrenergic innervation as demonstrated by DBH-immunoreactivity confirms observations performed with glyoxylic acid-induced histofluorescence. Our results are also consistent with known changes in regional concentrations of norepinephrine during development.

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264.12 EFFECTS OF NERVE GROWTH FACTOR ON RECOVERY FROM SPINAL CORD HEMISECTION. E. Eidelberg, J.T. Hansen and R. Pérez-Polo. Veterans Administration Hospital, San Antonio; Depts. of Surgery and Cellular and Structural Biology, UTHSC (San Antonio), Dept. of Biochemistry, UTMB (Galveston)

Lateral hemisection of the spinal cord produces ipsilateral paresis or paralysis and contralateral hypoesthesia, caudal to the lesion (Brown-Séquard syndrome). Both the sensory and motor deficits become greatly attenuated in the course of time, in humans and in experimental animals; the nature of the processes mediating the recovery is unknown. We attempted to modify it by local infusion of nerve growth (NGF) next to the segments (T6-T8) where the lesion was made. Most of the monkeys thus treated failed to recover any useful motor function with the initially paralyzed hindlimb, although the lesions themselves were not noticeably modified by the NGF. Animals infused locally with antiserum to NGF recovered at least as well as those receiving saline solution. Since NGF is known to promote the growth of catecholamine-containing axons, we studied the pattern of distribution of CA-fluorescent fibers and of boutons containing dense-core vesicles in cord segments distal to the hemisection. We will report on the consequences of NGF administration upon that pattern. Supported by grants from the Moody Foundation of Galveston, Texas and NIH RCDA KO4-HL-00680 to J.T.H.

264.13 SPINAL CORD CONTUSION IN THE RAT: I. PRODUCTION OF GRADED, REPRODUCIBLE, INJURY GROUPS. F. Harvey*, J. R. Wrathall and R. K. Pettegrew. Dept. of Anatomy, Georgetown University, Washington, DC 20007 and Dept. of Biology, Denison University, Granville, OH 43023

A weight drop (WD) technique was used to produce a contusive injury of the spinal cord in the rat. A restricted laminectomy was performed at T8 and the spinal column stabilized by means of clamps attached to the spinous processes of adjacent vertebrae. A 2.4 mm diameter impounder was lowered onto the dura and a 10 g weight dropped 0.0, 2.5, 5.0, 7.5, 10.0 or 17.5 cm onto the impounder. The functional deficit was assessed at 24 hr, 1, 2, 3, and 4 weeks after injury. Hindlimb function was evaluated according to a modified Tarlov scale and by the inclined plane test of Rivlin and Tator. Additional tests of reflex and complex behavior were employed to further characterize the functional deficit as described in the poster by Kerasidis et al. At 4 weeks after injury the rats were perfused and the spinal cord tissue processed for histopathological analysis. The results indicated that groups of rats (n = 10) subjected to the weight dropped from increasing height exhibited a graded functional deficit. With the modified Tarlov scale the deficit at 4 weeks ranged from mild in the 2.5 cm WD group where most (9/10) rats were capable of weight bearing and good, albeit abnormal, use of the hindlimbs in locomotion to severe in the 17.5 cm WD group where none (0/10) of the rats could use their hindlimbs for weight bearing and locomotion. Similarly, the mean inclined plane score decreased with increasing WD height. Histopathological results also indicated the production of graded lesions. The lesion in the 2.5 cm group involved some or all of the gray matter with most of the white matter remaining intact. In the 5.0 cm and higher WD groups, gray matter was not generally present at the epicenter. The rim of remaining white matter appeared to decrease in thickness with higher WD height and greater functional deficit. Based on the mean inclined plane scores at 4 weeks after injury, three groups of experimental animals were statistically distinguished corresponding to those with mild, moderate, and severe final functional deficit and produced by dropping the weight from 2.5, 5.0 and 17.5 cm respectively. The inclined plane scores (mean $\pm$ SEM) for these groups were 49.5 $\pm$ 1.2, 38.5 $\pm$ 3.1 and 26.0 $\pm$ 0.7 . The average functional deficit for these three experimental groups was reproducible in replicate experiments. It therefore appears feasible to use the WD technique to produce graded spinal cord injury groups in the rat.

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264.14 SPINAL CORD CONTUSION IN THE RAT: II. BEHAVIORAL ANALYSIS OF FUNCTIONAL NEUROLOGICAL IMPAIRMENT. H. Kerasidis*, K. Gale and J. R. Wrathall (SPON: L. Tolbert). Dept. of Anatomy, Georgetown University, Washington DC 20007.

A graded spinal cord injury in rats was produced by dropping a 10 gm weight from 2.5, 5.0, 10.0 and 17.5 cm onto the exposed dura at the T8 vertebral level. Groups of rats (n = 9 or 10) for each of these weight drop (WD) levels as well as unoperated and WD controls (0 cm) were subjected to extensive behavioral analysis that included tests of simple and complex reflexes (including responses to pain, touch and position) as well as spontaneous and evoked motor patterns. While several of the tests were well correlated with each other, no two tests correlated to such an extent that they could be used interchangeably. On the basis of the results from this analysis, a protocol for evaluating functional deficits following spinal cord injury in the rat was developed. The resulting Combined Behavioral Score (CBS), a measure of functional deficit, at 4 weeks after injury was closely correlated with the magnitude of mechanical injury ($r = 0.79$, $p<.01$). This relationship between functional deficit and WD height was also observed at 1, 2, and 3 weeks postoperatively. However, for all injury groups, there was a tendency for the CBS to decrease with time over the 4 week postoperative period. The scores obtained from animals in each injury group at 4 weeks were significantly lower than those obtained at one week when analyzed on a paired basis (t-test, $p<.01$). A similar analysis indicated no significant difference between scores obtained at 3 and 4 weeks.

A second experiment was conducted to determine whether the results were reproducible. The data obtained at 4 weeks post injury in the second experiment show that for a given injury magnitude, there were no significant differences between the mean CBS values of the replicate and the original experiments.

The use of multiple tests of both sensory and motor function allows a qualitative analysis of the nature of the deficits, which could be potentially useful in discriminating between different kinds of spinal cord injuries, and reduces the likelihood that an experimental artifact in any single testing procedure will significantly alter the results.

The qualitative and quantitative aspects of this behavioral analysis protocol now provide us with a functional profile that should be highly sensitive to detecting the effects of drug treatments on recovery of function in this rat model of spinal cord injury.

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264.15 SPINAL CORD CONTUSION IN THE RAT: III. MORPHOMETRIC ANALYSES OF ALTERATIONS IN THE SPINAL CORD. J.R. Wrathall and L.J. Noble. Dept. of Anatomy, Georgetown University School of Medicine, Washington, D.C. 20007.

Morphometric analyses were performed on sections of spinal cords which were injured 4 weeks previously by a weight drop (WD) technique. A 10 gram weight was dropped 0.0, 2.5, 5.0, 7.5, 10.0 or 17.5 cm onto the dura which was exposed at the T8 vertebral level. The lesion volume and length increased significantly from a 2.5 to a 17.5 cm WD injury. At the epicenter the cross-sectional area of gray matter was significantly reduced in a 2.5 cm WD injury as compared to the control, and in a 17.5 cm WD no gray matter remained. The cross-sectional area of white matter at the epicenter was significantly lower in all of the WD groups as compared to the control (0.0 cm) group. In general the area of the lesion at the epicenter enlarged as the WD height increased. The total area of the cord at the epicenter was significantly decreased in all of the WD groups as compared to the control group. Lesion volume, lesion length, and the dimensions of the tissue at the epicenter (lesion area, area of gray matter, and area of white matter) were correlated with the height from which the weight was dropped and the results from tests of motor and sensory functional deficit. A significant linear relationship exists between: 1. the height from which the weight was dropped and lesion volume ($r = 0.71$), lesion length ($r = 0.60$), gray matter remaining at the epicenter ($r = -0.62$), and white matter remaining at the epicenter ($r = -0.68$), 2. combined behavioral score at 4 weeks and lesion volume ($r = 0.68$), lesion length ($r = 0.68$), gray matter remaining at the epicenter ($r = -0.77$) and white matter remaining at the epicenter ($r = -0.91$). The area of the white matter at the epicenter is perhaps the best single measurement for characterizing the injury level, since it provides a simple but accurate depiction of the spinal cord's response to a crushing injury. With this single measurement data can be evaluated from a large sample of animals to reflect the response of a population to spinal cord injury. It is important, however, to recognize the limitations of a light microscopic analysis of residual white matter. The limited resolution at the light microscopic level precludes the identification of axonal pathologies which would be apparent at the ultrastructural level and can not distinguish an axon which is morphologically intact but physiologically disrupted. Further studies, using electron microscopy and tracer techniques, are being directed at analyzing the integrity of axons in the white matter after injury.

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264.16 ROLE OF CERTAIN ARACHIDONIC ACID METABOLITES IN POST-TRAUMATIC SPINAL CORD ISCHEMIA DEVELOPMENT. D.L. Wolf and E.D. Hall. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

The possible contribution of arachidonic acid (AA) metabolites to the development of post-traumatic ischemia was examined in the contused cat lumbar spinal cord. Injury to CNS tissue has been shown to activate membrane phospholipases resulting in the release of AA. AA metabolites, such as prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or thromboxane A_2 (TXA_2), are potent vasoconstrictors and/or platelet aggregators, and as such could promote ischemia development and tissue hypoxia. In addition, certain of the leukotrienes have vasoconstrictor actions in some vascular beds.

Complete dorsal L3 laminectomies were performed in pentobarbital-anesthetized cats and somatosensory evoked potential (SEP) conduction and white matter spinal cord blood flow (SCBF; H_2 clearance method) were monitored. Cats were treated i.v. 30 min prior to injury with either vehicle (VEH), the cyclooxygenase (CO) inhibitors ibuprofen (IBU; 10 mg/kg) or meclofenamate (MEC; 2 mg/kg), a TX synthetase inhibitor (TXI; U-63,557A, 10 mg/kg) or a lipoxygenase inhibitor (LPI; U-60,257, 10 mg/kg). A 500 g-cm contusion injury was applied to the exposed cord (dura intact) by dropping a 50 g weight 10 cm.

The mean pre-injury, pre-drug SCBF for all animals (N=24) was 12.37±0.74 (S.E.) ml/100 g/min. Only IBU injection significantly altered (paired t-test) pre-injury SCBF and caused a mean 3.1 ml/100 g/min increase. After injury, VEH-treated cats showed a progressive decline in SCBF in the injured segment. IBU and MEC (CO inhibitors) appeared to be effective in preventing post-traumatic ischemia development (table). However, the post-traumatic decline in SCBF following TXI or LPI treatment was similar to that seen in the VEH group.

MEAN POST-INJURY WHITE MATTER SCBF (ml/100 g/min)

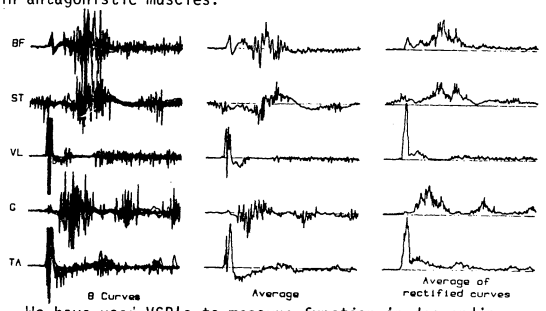
Group	N	30 Min	1 Hr	2 Hr	3 Hr	4 Hr
VEH	8	10.4	9.8	8.6	8.4	7.7
IBU	4	12.1	10.2	11.0	10.1	9.2
MEC	4	12.4	11.6	13.5	13.1	12.4
TXI	4	8.7	7.8	8.0	7.8	7.0
LPI	4	11.7	10.4	9.0	9.3	8.2

These results suggest that the potential injury-induced formation of vasoactive leukotrienes and TXA_2 in CNS tissue may not be as important as that of certain other AA metabolites in post-traumatic ischemia development. Accordingly, the cyclic endoperoxides and $PGF_{2\alpha}$ may be more likely mediators.

265.1 THE VESTIBULAR STIMULATED RESPONSE (VSR) TO FREE-FALL: FUNCTIONAL TESTING OF SPINAL CORD INJURED CATS. JA Gruner, W Young, Dept. Neurosurgery, NYU Med. Cntr., NY, NY 10016.

Vestibular mediated EMG responses initiated by sudden free-fall have been investigated in several species (Watt, DGD, J. Neurophysiol. 39:257-265, 1976). The VSR is ideally suited for testing residual spinal cord pathways after spinal cord injury in that it tests specific pathways; is sensitive, reliable, and generalizable across species, including man; requires no elaborate equipment or animal training; and is capable of assessing descending influences on spinal reflex pathways.

Intramuscular EMG recordings from the vastus lateralis (VL), biceps femoris (BF), semitendinosis (ST), gastrocnemius (G), and tibialis anterior (TA) were made in ketamine-sedated cats dropped 30 cm. The first 200 ms of the VSR is shown in the figure below. In general, there are at least two excitatory phases, E1 and E2, and one inhibitory period, I1. The E1 burst occurs between 20 and 40 ms following onset of free-fall, and is followed by I1, which has a variable duration. The onset of E2 depends on the length of I1. Excitatory activity tends to be reciprocally related in antagonistic muscles.



We have used VSR's to measure function in descending vestibulospinal tracts after experimental spinal cord contusions in cats. The responses correlated with the animals' ability to stand and/or walk. Small VSR's sometimes remained in paralyzed cats. Peripheral reflex stimulation can be used to enhance the sensitivity of the test, often revealing subthreshold vestibular effects on motoneurons.
Supported by American Paralysis Association #RC-83-02.

265.2 RECOVERY STRATEGIES IN HEMILABYRINTHECTOMIZED PATIENTS AFTER ACOUSTIC NEUROMA SURGERY. P.A.McKinley, B.W. Peterson, & C. Harf* Northwestern Dept. Physiol. & Rehab. Institute Chicago IL 60611

Although it is known that adaptation to deficits within the vestibulo-oculomotor pathways occurs, the mechanisms by which adaptation occurs is still under investigation. We have examined the Vestibuloocular Reflex (VOR) in hemilabyrinthectomized (HL) individuals that have recovered from acoustic neuroma surgery and have contrasted the responses of these patients to vestibular stimuli with those elicited in normal individuals.

HL were subjected to sinusoidal rotation (SIN) ranging from 0.01-2.5Hz in the dark while in the relaxed (R) state. R responses to SIN of 0.25-1.0Hz in a lit surround were used as baseline VOR. VOR was also examined for SIN of 0.25-1.0Hz under two mental sets: enhance by tracking an imaginary earth-fixed target (E) and suppress, by tracking an imaginary chair fixed target (S). Eye movements were recorded by DC electrooculography, desaccaded and differentiated to obtain the velocity of smooth phase eye movements (VSE). VSE was compared to chair velocity to obtain VOR phase and gain.

Ability to S or E VOR was excellent in all HL. Change in mental set modulated gain in a similar fashion as reported in normals (McKinley et al Neurosci. Abstr. 9:867 1983). However, R responses varied widely within the population of recovered HL. At one extreme, HL demonstrated high gain in both light and dark for SIN of 0.25-1.0Hz, and gain asymmetry and phase leads that were not significantly different from normals at 0.01-0.1Hz. In addition, drift at 2.5Hz was directed toward the lesioned side. At the other extreme, HL showed significant decrease in gain during SIN of 0.25-1.0Hz in light and dark, and large phase leads and gain asymmetries during low frequency rotation (0.01-0.1Hz) that were significantly different from normals.

These results indicate that HL use one of two recovery strategies: either reliance on voluntary modulation of the VOR or on plastic change in the VOR gain itelf. In essence, the latter allows a recalibration within the vestibular ocular neural network.
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265.3 FORCE-EMG RELATIONSHIPS IN SPASTIC PARETIC MUSCLES. M. J. Blaschak*, W. Z. Rymer, J. Marder-Meyer* (SPON:D. Zealear) Biomedical Engineering Program, Department of Physiology, Northwestern Univ., Sensory Motor Performance Program, Rehab. Inst., Chicago, IL 60611.

There are three possible sources of muscular weakness in spastic-paretic muscles of a brain-injured patient: loss of descending excitation to spinal neurons, muscle atrophy, and disorganization of motor output. Studies on animal models (Rymer, et al, Exp Brain Res, 37:93,1979) and on humans (Tang & Rymer, J N N P, 44:690,1981), reflecting lowered mean motor unit discharge rates, have lent support to motor output disorganization as being an important factor in the weakness of spasticity. A careful examination of the force-emg relationships and of the emg power spectrum recorded from spastic paretic muscles via surface electrodes will allow these three possibilities to be distinguished.

Surface emg's were recorded during constant force isometric contractions from twelve subjects with from mild to severe spastic hemiparesis. The subjects generated force against a load cell, placed at the wrist, through isometric contractions of the elbow flexors in response to a visual feedback cue. The force and the following emg measures were then recorded on-line at high resolution for 500 msec. epochs on a PDP 11/23 microcomputer: mean rectified emg, RMS, total power, peak power frequency, mean power frequency, median power frequency, peak power, zero crossings, and the number of peaks. In addition, the muscle fiber conduction velocity was determined through the use of dip analysis and cross-correlation measures.

Preliminary results indicate that approximately 50% of the cases demonstrate an increase in the slope of the force-emg relationship for the involved limb indicating that more emg was required to generate the same force. A more consistent difference between the non-involved and involved limb is found in the force-emg power relationship. In 83% of the cases, the involved-limb demonstrated an increase in the total power per unit force. Power spectrum analysis reveals a consistent shift of the spectrum to lower frequencies for the spastic muscles that is unassociated with any changes in the muscle fiber conduction velocity. This finding is consistent with the concept of lowered mean motor unit discharge rates as a mechanism for neuromuscular weakness.
Supported by NIH Grant #1 RO1 NS 19331

265.4 ELECTROMYOGRAPHIC ACTIVITY IN PARAPLEGIC INDIVIDUALS DURING STANDING IN KNEE-ANKLE-FOOT ORTHOSES. R.J. Jaeger. Pritzker Inst. Med. Eng., Ill. Inst. Technol., Chicago, IL 60616.

The knee-ankle-foot orthosis (KAFO) is presently the only widespread technique in rehabilitation medicine for standing and ambulation in paraplegia. It has been suggested that functional neuromuscular stimulation (FNS) has the potential to provide similar function in a limited number of paraplegic individuals (Vodovnik et al. CRC Crit. Rev. Bioeng. 6(2):63-131, 1981), but many problems remain to be solved. In attempting to use FNS to obtain functional muscle contraction for standing in upper motor neuron (UMN) paralysis, it is assumed that the stimulation is the only source of excitation. Remaining reflex activity ("spasticity") is assumed to make no significant contribution to the stimulated muscle or its antagonist. To test this assumption, remaining reflex activity present in UMN paralyzed muscle was studied during standing by KAFO, with hips in hyperextension, knees locked, and ankle joints unlocked to permit free rotation. Slow postural sway about the ankle in the antero-posterior direction was forced by using the upper extremities and a balance aid. Ankle angle was recorded by an electrogoniometer. Angular excursions were typically ± 4 degrees and velocity did not exceed 10 deg/sec. Surface EMG was recorded (gain 1000, bandwidth 60-10k Hz) from the gastrocnemius-soleus (GS) and tibialis anterior (TA). An attempt was made to elicit tendon tap responses from all muscles. Four subjects were tested. The results indicated two patterns of response. In one subject, phasic co-activation of GS and TA were observed during anterior sway (stretch GS). Both GS and TA were silent during posterior sway. In the other three subjects only low levels of constant EMG were seen in GS with no phasic relationship to sway. All subjects had brisk reflex responses in GS to tendon tap. The findings in three subjects are consistent with previous reports of a velocity threshold for the stretch reflex (Burke et al, J. Neurol. Neurosurg. Psychiat. 33:216-223, 1970). This is also consistent with observations in hemiparesis (Knutsson and Richards, Brain 102:405-430, 1979) in which individuals with marked spasticity during examination may demonstrate no spasticity in standing or walking. While there may be clinical cases with "severe spasticity" which are not appropriate, the present results suggest that remaining reflex activity in the muscles studied does not by itself preclude the use of FNS in this application.

265.5 **ACTIVE AND PASSIVE RANGE OF JOINT MOVEMENTS IN STROKE PATIENTS AFTER DESENSITIZATION OF THE SKIN.** M.A. Sabbahi, S. Roy*, C.J. De Luca and L.A. VanVolkinberg*. NeuroMuscular Research Lab., Childrens' Hosp. Med. Ctr., Spaulding Rehabilitation Hosp., Boston, MA 02115, and Liberty Mutual Research Ctr., Hopkinton, Ma 01748.

Previous studies in our laboratory have shown that desensitization of the skin, with topical anesthesia, result in substantial changes in movement parameters in patients affected with stroke. In the present study, desensitization of the skin was tested for its effect on the motor output and muscular rigidity in upper and lower limb joints.

Active and passive range of movements (ROM) were measured, using coventional methods, before and after a topical anesthetic or a placebo was applied to the skin of the affected limb in stroke patients (research design was discussed in SN abstract 298.10, 1983). Patients were then selected randomly to receive a physical therapy program combined with either the topical anesthetic or a placebo spray for one month. In the second month of the treatment program the spray was changed so that each patient was administered an active and a placebo spray during an equal amount of time. At the end of the two-month period, the tests were repeated and the changes were compared .

Results show that active as well as passive ROM substantially increased immediately post anesthesia. In many patients, this effect was more apparent in proximal than distal joints. The increased active ROM post anesthesia was also measured in those patients who demonstrated no change in passive ROM. Movement patterns shifted toward normal synergy. All these effects increased during the one-month period of the topical anesthesia program. No measurable changes were noticed post placebo or after the one-month period of placebo program. These results indicate that reduction of cutaneous receptor afferent discharges may reduce muscular rigidity (as tested by passive movements) and may increase motor output to contracting muscles in stroke patients. This interpretation is consistent with that of our previous studies on H and Achilles tendon reflexes. The improved performance was more evident in proximal than in distal muscle groups. This outcome may prove useful for augmenting the functional capability of stroke patients. (Supported by Liberty Mutual Insurance Co.)

265.6 FINE FORCE AND POSITION CONTROL OF SELECT LIMB AND OROFACIAL STRUCTURES IN THE UPPER MOTOR NEURON SYNDROME. S.M. Barlow, Speech Motor Control Laboratories, Univ. of Wisconsin, Madison, WI 53705 and Speech Physiology Laboratory, Boys Town National Institute, Omaha, NE 68131.

Direct motor cortex outputs have been deemed especially important in controlling motor neurons initially recruited for precise adjustments in force (Fromm, C., Adv. Neurol., 39:329, 1983) and displacement (Fromm, C. and Evarts, E., Neurosci. Lett., 5:259, 1977) underlying skilled motor behavior. A central thesis of the present study is that speech and other precise motor skills exhibited by humans are regulated by a phylogenetically sophisticated neocortical control system. Damage to the cerebrum including portions of the motor cortex was hypothesized to degrade fine force and fine position control, especially in those structures involved in speech and manipulation. Given these considerations, submaximal isometric force and precise position control of the upper lip, lower lip, tongue, jaw and wrist were investigated in five adults with a congenital form of the Upper Motor Neuron-Syndrome (UMN-S) with presumed motor cortex involvement and in normal subjects matched for age and sex. Subjects were asked to make rapid and accurate adjustments in fine levels of force and position under visual feedback. Force and displacement signals were sampled on-line with a PDP-11/44 computer and quantified in terms of the rate of force change, displacement velocity, rise time and target end-point.

A fundamental pathophysiologic feature of UMN-S subjects was the impaired ability to rapidly adjust fine levels of force and position in the upper lip, lower lip, tongue, jaw and wrist. Absolute force and position end-point accuracy was relatively well preserved. Structures normally capable of finer motor control manifest disproportionately greater deficits in the ability to generate precise forces and displacements. For example, deficits in fine force and position control were consistently greater in the lower lip as compared to the upper lip. These findings have obvious implications for the execution and performance of fine motor skills, e.g., speech and manipulation, since the presumed muscle forcing functions are thought to involve submaximal force generation.

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265.7 ABNORMAL ANTAGONIST MUSCLE ACTIVITY OCCURS IN ELBOW FLEXIONS DURING CEREBELLAR DYSFUNCTION. D. Flament*, T. Vilis and J. Hore (SPON: G. Ferguson). Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada, N6A 5C1.

After an arm perturbation, monkeys with lateral cerebellar dysfunction show cerebellar intention tremor (Vilis and Hore, 1980). From the onset of the perturbation the first abnormality associated with this tremor was changed timing of EMG activity in the antagonist muscle. While under normal conditions antagonist muscle activity could lead muscle stretch, during cerebellar dysfunction it followed antagonist muscle stretch i.e. it was due to abnormal stretch reflexes. Disorders also occur in voluntary movements during cerebellar dysfunction. The present study was undertaken to determine whether these disorders are also due in part to abnormal antagonist activity resulting from stretch reflexes.

Five Cebus monkeys made fast and accurate elbow flexions with a triphasic EMG pattern. During cooling through probes implanted lateral and medial to the dentate nucleus all monkeys developed a deflection in movements such that the movements had bimodal rather than unimodal velocity profiles. This deflection was most prominent when a constant force was placed on the handle that loaded the antagonist muscle. It was decreased or abolished when a 100 gm mass was added to the handle.

The abnormal deflection was associated with either earlier onset or increased magnitude of antagonist muscle activity. The abnormally early antagonist burst had a minimum latency of 30 ms from start of movement. In addition it was influenced by mechanical changes applied to the limb. It often appeared when a constant force was applied that loaded the antagonist. It was decreased in amplitude or absent when the constant force loaded the agonist. It was also decreased in amplitude or abolished when a 100 gm weight was added to the handle. With this added mass there was increased agonist activity and decreased initial velocities compared to normal conditions.

These findings indicate that the deflection in movements during cerebellar dysfunction is initiated by abnormal antagonist activity. The latency of this abnormal antagonist burst and its characteristics under different mechanical conditions suggest it may result from abnormal stretch reflexes.

Vilis, T. and Hore, J. (1980) J. Neurophysiol. 43, 279-291.

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265.8 A M.P.T.P. PRIMATE MODEL OF PARKINSON 'S DISEASE : AN E.M.G. STUDY. D. Doudet*, C. Gross*, P. Lebrun-Grandié* and B. Bioulac. Lab. de Neurophysiologie, Groupe Motricité, Univ. de Bordeaux II, 146 rue Léo Saignat, 33076 BORDEAUX CEDEX (FRANCE).

Recently, the development of irreversible parkinsonism in human and monkey after i.v. or i.p. administration of 1-methyl-4-phenyl-1, 2, 5, 6 tetrahydropyridine (M.P.T.P.) has been reported (Burns et al., Proc. Natl. Acad. Sci. U.S.A., 80 : 4546, 1983 ; Langston et al., Brain Res., 292 : 390, 1984). To appreciate the validity of this new primate model of Parkinson's disease, we have examined several parameters of extension (X) and flexion (F) movement of the forearm and the electromyographic (E.M.G.) activity of the agonist/antagonist couple in normal and lesioned monkeys. **Methods.** Two monkeys were trained to perform a rapid elbow movement of X and F in response to an auditory cue. The E.M.G. was obtained from biceps and triceps with intramuscular electrodes. E.M.G. activity, full-wave rectified and integrated, and movement parameters (behavioural reaction time (RT), movement time (MT) and maximal velocity) were analyzed from 500 msec before to 1500 msec after the beginning of the auditory signal. Three doses of M.P.T.P. (2 mg/kg each) were intraperitoneally injected at 2 h intervals. Recording sessions began one week after the drug administration. **Behavioural observations :** After 2 doses of M.P.T.P., increasing bradykinesia was observed. The two monkeys became akinetic, usually sitting hunched over in a flexed posture. They exhibited a generalized increase in tone. The animals showed an apparent difficulty in performing the arm movement and, sometimes, froze in the middle of its execution. Tremor was never seen. Vocalization was diminished. **Movement parameters and E.M.G. activity.** Mean values of RT and MT were significantly increased (26 % for RT ; 111 % for MT) for both X and F movements ($p < 0.05$). There was also a reduction (12 %) of the mean maximal velocity of movement. In comparison with the normal E.M.G. activity, we observed a constant disorganization of the E.M.G. activity in the agonist/antagonist muscles in lesioned animals.

Conclusion. Perturbations in behaviour, E.M.G. and movement parameters which we observed in our monkeys look like the characteristic features reported in parkinsonian patients (Hallett et al., J. Neurol. Neurosurg. Psychiat., 40 : 1129, 1977 ; Evarts et al., Brain, 104 : 167, 1981). These preliminary results, added to the previously histological and behavioural reports reinforce the assumption that use of MPTP provides a useful model for studying Parkinson's disease.

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265.9 SIMPLE REACTION TIME, REPETITION RATE, AND RATE MANIPULATION IN SPEECH IN PARKINSON'S AND HUNTINGTON'S DISEASE. C. L. Ludlow, N. P. Connor*, and C. J. Bassich*. Speech Pathology Unit, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, MD 20205.

Slow reaction times occur in several diseases affecting the basal ganglia. Patients with Parkinson's disease (PD) and Huntington's disease (HD) were contrasted on measures of speech reaction time to an acoustic signal, rate, repetition and offset. Normal inter-relationships between reaction time and speech rate, syllable offset time and speech rate, rate of laryngeal adduction/abduction and repetition rate, and between reaction times of various articulators were also examined. The purpose was to determine whether different aspects of speech movement patterning are independently affected in different diseases of the basal ganglia. The following measures were made from spectrographic analyses of the speech productions of the patient groups and age and sex matched normal controls: 1) simple reaction time for speech movements involving the larynx alone, larynx and lips and larynx and tongue; 2) repetition rate and maintenance of rate; 3) alterations in speaking rate, and 4) control of syllable offset. Neither group was impaired in simple laryngeal reaction time or the maintenance of rate during syllable repetition. Both groups were impaired in maximum rate of repetition. HD patients had slower reaction times than normal for speech movements requiring laryngeal and lip coordination and were excessively slow on all repetitive and speech rate measures. PD patients were particularly slow on movements requiring rapid changes between laryngeal adduction and abduction. The normal relationships between speech reaction time and rate and between rate of laryngeal adduction-abduction and repetition rate were not found in either patient group. Only the HD group retained some of the normal relationships between the reaction times of the various articulators. Both groups demonstrated the normal relationship between syllable offset time and speech rate. Thus, impairments in rate and control of repetitive movements were independent of speech reaction time in the two patient groups. Also, the pattern of relationships found between performances on the various tasks differed in the two patient groups, indicating that different diseases of the basal ganglia have selective and different effects on speech motor control.

265.10 SENSORIMOTOR DYSFUNCTION IN PARKINSON'S DISEASE: OBSERVATIONS FROM A MULTIARTICULATE SPEECH TASK. V.L. Gracco and J.H. Abbs*. Speech Motor Control Labs., Waisman Center, Univ. of Wisconsin, Madison, WI 53705-2280.

Recent studies have implicated the basal ganglia in motor programming and pathogenesis of movement aberrations associated with Parkinson's disease (Marsden, C.D., Neurol., 32:514, 1982; DeLong et al., Human Neurobiol., 2:235, 1984). Specifically, basal ganglia output has been hypothesized to scale the amplitude of limb movements through its influence on EMG magnitude. Additionally, patients with Parkinson's disease frequently exhibit difficulty in producing large amplitude limb movements. These observations, however, are limited to single joint adjustments. Investigations of multiarticulate movements may provide additional insight into the pathogenesis of movement aberrations associated with Parkinson's disease.

In the present study we investigated the EMG and kinematic compensatory responses in upper and lower lips of Parkinson subjects to lower lip perturbations during speech. Inferiorly directed loads were applied to the lower lip via a torque motor. Load magnitudes were 35 and 55 grams.

Comparison of autogenic and nonautogenic EMG and movement changes with load onset time revealed differential compensatory patterns. Compensatory response from the autogenic lower lip was reduced in gain relative to normal. Nonautogenic upper lip responses, however, were increased. Although autogenic responses were reduced, responses were scaled to load magnitude. That is, the slope of the regression line relating movement compensation to perturbation displacement was greater for the 55 gram load relative to the 35 gram load. The ability to modulate output in a proportional manner indicates that sensory input is relatively unaffected. Finally, compensatory response latencies were significantly longer, often 100 msec, with an absence of mid latency (35-55 msec) responses previously reported (Abbs & Gracco, J. Neurophysiol., 51(4):705-723, 1984).

Overall, results reflect a reduced ability to scale orofacial movements consistent with a motor deficit associated with basal ganglia lesion. In contrast to single joint movement, increased reliance on nonautogenic responses reflect a functional adaptation in which available degrees of movement freedom are used to accomplish the multiarticulate motor task. Results will be discussed in terms of the differential effects of basal ganglia lesions on the programming and execution of coordinated speech movements. Research supported by grants from NIH (NS-13274 and HD-03352).

265.11 PARKINSONIAN RESTING TREMOR AND ITS RELATIONSHIP TO MOVEMENT INITIATION DELAYS. C.J. Hunker* and J.H. Abbs* (SPON: F. Graham). Speech Motor Control Labs., Waisman Center, Univ. of Wisconsin, Madison, WI 53705-2280.

Increases in reaction times have been substantiated empirically in the limbs of some Parkinson patients. It has been hypothesized that the movement initiation delays in Parkinson patients with resting tremor may be due to an inability to initiate a voluntary muscle contraction until it coincides with the involuntary excitatory EMG burst of the resting tremor cycle in agonist muscles. The experiment reported here offers strong support for this hypothesis. Agonist EMG and movements from the lips, tongue, jaw, and index finger were recorded from two groups of Parkinson subjects (with and without resting tremor), as well as from a normal control group. The motor task included the generation of movements from rest under both self-paced and reaction time conditions.

In each structure manifesting tremor, an in-phase relationship between the resting tremor and the onset of voluntary movement was observed. It was consistently demonstrated that movement related EMG activity was in synchrony with the excitatory agonist phase of the resting tremor. In parallel, reaction time latencies were significantly longer in the tremorous Parkinson patients than in the nontremorous Parkinson or normal counterparts. It was found that both the neural (NRT) and mechanical (MRT) response components contributed to the increased reaction time in the tremorous Parkinson patients. The elongated NRT was directly related to the synchronization of voluntary muscle activity with the excitatory phase of the tremor cycle, while the elongated MRT was hypothesized to result from characteristics intrinsic to the tremorous muscles. Research supported by grants from NIH (NS-13274, HD-03352).

265.12 RESPONSE PROGRAMMING CAPABILITIES IN PARKINSON PATIENTS AS ASSESSED BY CHOICE REACTION TIME. M.S. Newton* and D.C. Shapiro (SPON: G.P. MOORE). Motor Control Lab., Dept. of Kinesiology, UCLA, Los Angeles, CA 90024.

It is hypothesized that the deficit exhibited by Parkinson patients is an inability to program responses in advance (Flowers, K. Brain, 99, 269-310, 1976). This hypothesis was examined utilizing a reaction time paradigm. Klapp et al. (J. Mot. Behav., 6, 263-271, 1974) hypothesized that choice reaction time (CRT) should reflect programming time and as complexity of a response increases CRT should also increase. Simple reaction time (SRT) should not reflect programming time, since subjects know the response in advance of the stimulus and can hold the program in memory until the stimulus appears. Klapp et al. demonstrated that subjects performing a morse code "dit" response, a quick press and release of a short duration reacted faster than when performing a "dah" response, a quick press, hold, and release of a longer response duration. Thus, as response complexity increased (dah), CRT also increased. This paradigm was employed on Parkinson patients and a normal group of the same age. If Parkinson patients cannot program movements in advance, task complexity will not be reflected in the CRT.

Twelve subjects diagnosed as having Parkinson's syndrome and twelve non-Parkinson subjects volunteered. The mean age of the Parkinson group was 70 and the normals was 69. Subjects performed key-press responses of either 100 (dit) or 300 (dah) ms with the index finger of the preferred hand. Subjects were informed to react as quickly as possible following the stimulus onset in both SRT and CRT situations.

A significant movement time (MT) difference between dit and dah responses for both simple and choice reaction times was found, which validates our MT manipulation. There was no significant SRT difference between dit and dah key presses as predicted. There was, however, a significant difference between normals and Parkinson patients, with patients demonstrating significantly longer latencies than normals. The critical comparison was for CRT. There was a significant difference between the dit and dah responses for both groups and no significant difference between Parkinsons and normals. The results suggest that Parkinson patients can program as well as normals as assessed by the CRT differences. More complex responses require greater programming time for both normal and Parkinson patients. (Supported by a UCLA Academic Senate Grant).

265.13 Tolerance of the Peripheral Nervous System of Dogs to Intraoperative Radiation, A.M. DeLuca, T.J. Kinsella*, W.F. Sindelar*, R. Terrill*, K. Kranda*, and A. Mixon*, Radiation Oncology and Surgery Branches, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205.

The peripheral nervous system is considered to be resistant to high dose (6000-7000 rad) conventional fractionated external beam radiation therapy. When peripheral nerves are injured with external radiation, clinical signs are not manifested for months (usually more than 9 months) to years following treatment. In preliminary clinical trials of intraoperative radiotherapy (IORT) using 2000-3000 rad, three patients developed clinical signs of peripheral nerve injury within 4-6 months following IORT.

To further investigate the tolerance of peripheral nerves to IORT, the right lumbro-sacral plexus (L4-S1) of twenty four pure-bred Foxhounds were irradiated during surgery with single large doses ranging from 2000 to 7500 rad in 500 to 1000 rad increments. The irradiations were performed with an 11 MeV electron beam and the field was outlined by a 9 cm beveled circle lucite treatment cone. The cone was inserted into the groin to retract the viscera and to define the treatment area. The perimeter of the cone was encased in a stainless steel shield and the viscera were protected with lead.

Electromyograms, nerve conduction tests and neurological examinations were performed on all the dogs at monthly intervals in order to access the course of the nerve damage.

Using hind limb limp as an end-point, there was a linear time-dose relationship with the highest doses (6500-7000 rad) having an effect between 2 and 15 weeks and the lower doses (2000-3000 rad) at 40 weeks or later. The clinical picture is one of lower motor neuron disease of the leg. Muscle stretch reflexes are lost or reduced. There is progressive sensory loss or hyperesthesia. Muscle wasting and often fasiculations are seen. The sham treated dogs show normal hind limb function.

One year after treatment or when paralysis became severe, one dog in each dose group was sacrificed. Preliminary histological examination of the neural tissue reveals Wallerian degeneration of the fibers with fragmentation of axons and myelin sheaths. The remaining dogs are part of a long term follow-up study.

265.14 FATIGUE OF NORMAL CANINE DIAPHRGAM DURING INTRAMUSCULAR STIMULATION. D.K. Peterson*, M.L. Nochomovitz* , and J.T. Mortimer (SPON: R. Grubbs). Applied Neural Control Lab., Case Western Reserve Univ., Cleve., OH 44106.

One application of intramuscular diaphragm pacing is as a short term respiratory assist device. This serves as one motivation for study of the fatigue characteristics of diaphragm pacing as a function of stimulus parameters under acute conditions. A second motivation is to establish stimulus parameters to be used in long term chronic studies.

Six adult mongrel dogs (10-15kg) anesthetized with sodium pentobarbitol (30mg/kg iv) were studied. Monopolar coiled wire stimulating electrodes were implanted bilaterally near (1-2 cm) the trifurcation of the phrenic nerve. Transdiaphragmatic pressure measurements were used as an index of diaphragm force production. Bifilar recording electrodes implanted in the lateral and/or crural diaphragm 2-4 cm from the costal margin were used to measure compound muscle action potentials (MAPs) evoked by intramuscular stimulation. Retrograde compound nerve action potentials (NAPs) were recorded from the 5th root of the phrenic nerve in the neck using tripolar cuff electrodes. Supramaximal stimulus pulses (20 mA, 100 usec) were applied in either 20 or 40 Hz bursts lasting 1 second at a rate of 50 bursts per minute for periods up to six hours. Force versus frequency measurements were made against an occluded airway before, after, and during recovery from fatigue.

Our studies indicate that 20 Hz stimulation may be continued for up to 4 hours without signs of fatigue despite the high rate and large duty cycle used. However, 40 Hz stimulation induces a 50 % drop in diaphragm force production within a 1 hour period of pacing. Concurrent with the drop in diaphragm force is a reduction in amplitude and a broadening in width of the evoked diaphragm MAP. The rectified and integrated MAP also declines with the drop in diaphragm force. No significant changes were found in the retrograde phrenic NAP throughout the studies. Recovery of evoked diaphragm force and MAP is complete after 30 minutes to 1 hour of rest on a mechanical ventilator. The rate of diaphragm force and MAP fatigue is greater in subsequent pacing periods.

These results suggest any site(s) of fatigue during the first 4-6 hours of intramuscular diaphragm pacing lie at or beyond the neuromuscular junction and not within the excitation and propagation elements of the phrenic nerve.

265.15 A TECHNIQUE FOR COLLISION BLOCK OF PERIPHERAL NERVE: FREQUENCY AND PULSE CHARACTERISTIC DEPENDENCE. J.D. Sweeney*, J.T. Mortimer, G.G. Naples* and T.J. Crish*, Applied Neural Control Lab., Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106.

We have reported (J.D. Sweeney and J.T. Mortimer, Soc. Neurosci. Abstr. 9: 1038, 1983) initial results on the development of an asymmetrically shielded two electrode cuff (ASTEC) for collision block of peripheral nerve. This modified bipolar cuff electrode can elicit a neural collision block through generation of antidromic unidirectionally propagated action potentials (AUPAP). Such a collision block could be used to suppress spasticity of the external urinary sphincter in patients with detrusor-sphincter dyssynergia.

Following our acute animal study of AUPAP generation using single monophasic regulated-current rectangular pulses with exponential trailing phases we have investigated ASTEC dependence upon the frequency of both monophasic and balanced-charge biphasic (BCB) stimulation. In three adult cats with ASTECs acutely placed on the medial gastrocnemius (MG) branch of one sciatic nerve monophasic stimulation generated AUPAP with a maximum frequency ranging from 40 to 100 Hz before bidirectional propagation occured. BCB stimulation, which would be preferred in chronic implantation for electrochemical reasons, produced AUPAP at a maximum frequency ranging from 10 to 50 Hz.

We have also studied in three adult cats the effectiveness of ASTEC generation of AUPAP on a larger nerve trunk, the greater sciatic. Using single monophasic regulated-current pulses a cuff geometry "scaled up" from that of the MG cuff has been found that effectively produces AUPAP while minimizing overall cuff length. By monitoring MG force and tibial nerve ENG we have found that the current threshold for AUPAP generation is lower for the more superficially located MG nerve fibers than the threshold for the deeper sciatic fibers that continue into the tibial nerve. In three cats we have also found that trains of monophasic stimuli can generate AUPAP at a maximum frequency ranging from 40 to 50 Hz while BCB stimuli can only reach 10 to 30 Hz. At the BCB stimulation frequency where bidirectional propagation is elicited the undesired orthodromic excitation occurs at the onset of the secondary charge-balancing pulse. We have designed a new stimulator that smoothly shifts from the exponential trailing phase of the primary pulse into the secondary pulse. In an initial test this waveform enabled an increase in BCB maximal rate from 30 Hz to 37 Hz.

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265.16 MUSCLE PLASTICITY INDUCED BY ELECTRICAL STIMULATION: A COMPARISON OF STIMULATION PARADIGMS AND HOURS/DAY OF STIMULATION. A.S. Ferguson*, H.E. Stone*, J.T. Mortimer, M. Burke*, and E. Tisdale* (SPON: L.F. Dell'Osso). Applied Neural Control Lab., Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106.

Physiological, metabolic and biochemical properties of muscle alter in response to electrical stimulation. We have previously compared (Soc. Neurosci. Abstr. 9: 1038, 1983) the effects of two different stimulation paradigms on muscles stimulated for 24 hours/day. More recently we have investigated the effects of these same paradigms on muscles stimulated for 8 hours/day and compared the biochemistry of the two groups of muscles.

Tibialis anterior muscles of cat hind limbs were stimulated supramaximally for 90 days with 100 usec balanced biphasic current pulses. One limb received 10 Hz continuous stimulation; the other limb received a 30 Hz burst paradigm modified to produce an average stimulation rate of 10 Hz. Both limbs were stimulated for either 8 or 24 hours per day.

Muscles stimulated for 8 hours/day show less complete fiber conversion than muscles stimulated for 24 hours/day. The latter exhibited a uniformly light myofibrillar ATPase stain; 8 hour stimulated muscles contained a large percentage of dark staining fibers, although there were more light staining fibers compared to control muscle. All stimulated muscles stained uniformly dark for NADH, indicating oxidative metabolism. Muscle mass in the 24 hours/day muscles was significantly greater using the 30 hz burst compared to the 10 Hz paradigm; no difference in muscle mass resulted between the two paradigms following stimulation for 8 hours/day. Endomysial fibrosis, increased nuclei and inflammatory response, all apparent in the 10 Hz/24 hour muscles, were absent in the 8 hour stimulated muscles.

In accord with the myofibrillar ATPase stain, muscles stimulated for 8 hours per day exhibited contraction times longer than control, but shorter than 24 hour stimulated muscles. Regardless of the hours/day of stimulation, 30 Hz muscles had a lower twitch force but larger tetanic force, resulting in a smaller twitch/tetanus ratio than that found in the 10 Hz muscle. Following one hour of stimulation at their respective paradigms, all 30 Hz muscles exhibited a larger force-time integral than the 10 Hz muscles. The 30 Hz paradigm is more effective in adapting muscle to applying force for longer periods of time, indicating this will be a more useful stimulation paradigm for altering bone structure as may be required in for correction of a scoliotic spine.

We are attempting to correlate biochemical changes in the muscles (using SDS gels) with the above physiological and histochemical changes. No differences have been found in the contractile proteins of muscles stimulated for the same number of hours per day, despite the differences found in the other muscle properties. In the 8 hour muscles, for both 10 and 30 Hz paradigms, we see a very prominent light chain band at 27 K Daltons. In 24 hour stimulated muscle, the prominent band occurs at 24.5 K Daltons.

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265.17 *EFFECT OF MANUAL AND VOLUNTARY RANGING ON MUSCLE STIMULATED UNDER RESTRICTED LENGTH CONDITIONS.* H.E. Stone*, A.S. Ferguson*, J.T. Mortimer and E. Tisdale* (SPON: C. van den Honert). Applied Neural Control Lab, Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106.

The effects of periodic ranging (large changes in muscle length) on muscle stimulated under restricted length conditions have been investigated. Electrical stimlulation in combination with bracing has been proposed as a possible treatment for scoliosis (lateral curvature of the spine). But, it has been found that cat muscle subjected to repeated stimulation under restricted length condtions undergoes severe changes which may be deleterious (Kobetic, Mortimer, Roessmann). If ranging can prevent these changes from occurring, patients who are being braced and stimulated simultaneously may be advised to perform ranging exercises.

In 9 cats, the tibialis anterior muscle of one hind limb was stimulated supramaximally with 100 usec balanced biphasic pulses, while being held at a restricted length with a modified Thomas splint. The stimulation (consisting of a continuous 10 Hz or a 30 Hz burst paradigm with an average frequency of 10 Hz) and bracing was applied continuously for either 23 or 8 hours per day, with an interruption (1 or 16 hours, respectively) during which no stimulation was applied, and the limb was debraced and ranged manually. This procedure was continued for 90 days. The tibialis anterior of the contralateral limb in each cat served as the control.

Muscles stimulated for 23 hr/day (with a 10 Hz or 30 Hz burst paradigm) under restricted length conditions showed marked changes. Histochemical analysis showed uniform fiber type: fibers stained dark for NADH (indicating high oxidative enzyme activity) and stained light for myofibrillar ATPase. Both mean fiber area and muscle mass were reduced. Endomysial fibrous tissue proliferation was found, along with motheaten and central core fibers. Physiological data showed longer twitch widths and decreased active twitch force and tetanic force. Although the experimental muscles had greater resistance to fatigue, the integral of the isometric force produced during a one hour test was not increased over control.

Muscles stimulated with a 30 Hz burst paradigm under restricted length conditions for 8 hr/day showed no deleterious changes. Fibers stained uniformly dark for NADH. Mean fiber area, muscle mass, endomysial fibrous tissue and mitochondrial distribution were all normal. Longer twitch widths were measured but active twitch force and tetanic force were not reduced. Experimental muscles were more fatigue resistant and produced larger force-time integrals. Because of the absence of deleterious changes and the increased ability of these muscles to apply large forces for longer periods of time, stimulation with a 30 Hz burst paradigm under restricted length conditions for 8 hr/day could be useful in the treatment of a scoliotic spine.

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265.18 NEURAL ACTIVITY EVOKED BY PROLONGED STIMULATION WITH CHRONICALLY-IMPLANTED INTRACORTICAL MICROELECTRODES. D. McCreery, Leo Bullara* and W. F. Agnew. Neurological Research Lab., Huntington Med. Research Institutes, Pasadena, CA 91105.

Stimulating microelectrodes fabricated from Pt30%Ir are implanted in the precruciate gyrus of adult cats under general anesthesia. The electrodes are 1500 μ in length and have smooth, planar tips with geometric areas of 20 ±0.5 X $10^{-6}cm^2$.

Three weeks after implantation of the microelectrodes an insulated stainless steel wire is inserted by stereotaxis into the ipsilateral medullary pyramidal tract, to monitor the neural activity evoked by the intracortical microelectrodes during continuous stimulation, which is conducted with the cat awake and able to move about freely. The intracortical stimuli are charge-balanced, symmetrical, controlled-current, cathodic-first pulse pairs, 200 μsec/ph in duration, at 20 pps. The pulses are 20 to 3200 $\mu C/cm^2$. Stimulation is continued for 24 hrs, or for 23 hrs/day for one week. At intervals of 15 min to 1 hr, the computer-averaged compound action potential (CAP) is recorded from the pyramidal tract. During continuous intracortical stimulation at 20 to 40 μamp, the early components of the CAP remain quite stable, although some dimunition over time was observed, as well as hr to hr variations. These early components of the CAP probably reflect action potentials evoked directly in cortical neurons by the electrical stimulus. After 24 hrs of continuous stimulation, the threshold of the early component of the CAP is usually not elevated above the prestimulus value of 10 to 15 μamp. Late components of the CAP, which probably reflect neural activity evoked transsynaptically by intracortical stimulation, undergo greater attenuation, often within two hrs after the start of continuous stimulation. This reduction is reversible, but recovery may require up to 48 hrs. After stimulation for 24 hrs at 80 μamp (800 $\mu C/cm^2$), the threshold of the early component of the CAP may be elevated temporarily. However, even after stimulation at 80 μamp for 1 week, the elevation is temporary and reverses within a few days. In contrast, stimulation for only 24 hrs at 320 μamp produces profound elevation of the threshold of the early as well as the late components of the CAP. This elevation persists for at least 7 days after the end of stimulation. This is consistent with histological findings that such intense stimulation damages neuronal elements adjacent to the tip of the microelectrodes. Supported by: NIH Contract NO1-NS-3-2359.

265.19 MORPHOLOGICAL CHANGES FOLLOWING ELECTRICAL STIMULATIONS WITH INTRACORTICAL MICROELECTRODES. W. F. Agnew, T. G. H. Yuen*, L. A. Bullara* and D. B. McCreery. Neurological Res. Lab., Huntington Medical Research Institutes, Pasadena, CA 91105

Microelectrodes having geometric surface areas of approximately 20 x $10^{-6}cm^2$ were fabricated from either Pt30%Ir or pure iridium and implanted intracortically in the pre- or postcruciate gyrus of adult cats. A high valence oxide film was grown on the iridium electrodes by repeated cycling (activation) in vivo. Three weeks following implantation the electrodes were pulsed continuously for 24 or 160 hrs with cathodic-first, biphasic pulses using a pulse duration of 200 μsec per phase, 20 pps and pulse amplitudes of 10-320 μA (charge densities of 100-3200 $\mu C/cm^2 \cdot ph$).

Following stimulation for 24 hrs or 1 week (23 hrs/day for 7 days), the animals were anesthetized and perfused via the ascending aorta with either 10% formalin or a mixture of 3% glutaradehyde and 2% paraformaldehyde for light and electron microscopy, respectively. At 3 weeks following surgery, control (non-pulsed) platinum-iridium or pure iridium electrode sites showed moderate connective tissue ensheathment, mild gliosis, inflammatory cell infiltration around subdurally situated electrode array matrices (dacron mesh) and electrode tracks. Only at extremely high charge densities (3200 $\mu C/cm^2 \cdot ph$) was neural damage associated with Pt30%Ir electrodes. The neural damage was seen in the form of neuronal loss, axonal degeneration, necrosis and lipid-laden macrophages. However, neurons adjacent to these electrodes were activated by charge densities as low as 100 $\mu C/cm^2 \cdot ph$ as indicated by evoked potentials from pyramidal tract recordings.

Neural damage was not observed following pulsing with activated iridium electrodes at any charge density up to 3200 $\mu C/cm^2 \cdot ph$ and the neuronal activation threshold was essentially the same as for the Pt30%Ir. Thus, pure iridium microelectrodes appear to be the superior of the two metals for intracortical stimulations.

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265.20 SCANNING ELECTRON MICROSCOPE COMPARISON OF PLATINUM-30% IRIDIUM AND PURE IRIDIUM MICROELECTRODES USED FOR ELECTRICAL STIMULATION OF BRAIN CORTEX. T. G. H. Yuen*, L. A. Bullara*, D. B. McCreery and W. F. Agnew (SPON: D. Jacques). Neurological Research Laboratory, Huntington Medical Research Institutes, Pasadena, CA 91105.

The use of pure iridium for electrodes used in neural stimulation takes advantage of a property not shared by platinum-iridium alloys; the ability to inject charge by oxidation and reduction of a surface layer of high valence iridium oxide. Following initial formation of the oxide, continued oxidation and reduction of the oxide layer is not accompanied by erosion of the underlying metal matrix. In vitro experiments have shown that electrodes having an uninsulated working surface area of 20 x $10^{-6}cm^2$ can be covered by the oxide layer "activation" and are able to inject about 600,000 pC of charge. It has been demonstrated that only 4,000 pC/ph are needed to evoke action potentials in cortical neurons. A high-valence oxide film can be grown more conveniently on the electrodes in vivo immediately after surgical implantation. This is accomplished by repeated potential cycling at a frequency of 1/sec for 20 to 100 seconds between ± 0.8 volts against a large iridium counter electrode implanted in the temporalis muscle. Performing the activation process in vivo is more convenient and does not result in any histologically discernible tissue damage. The levels of charge injection used in the experiments were 100 to 3200 $\mu C/cm^2 \cdot ph$ for 24 hrs (Pt30%Ir) and 200-3200 $\mu C/cm^2 \cdot ph$ for 24 hrs (pure Ir). Stimulations were carried out with charge-balanced pulses using 200 μsec/ph pulse duration and 20 pps. Pulse amplitude was adjusted to obtain charge densities of 200 to 3200 $\mu C/cm^2 \cdot ph$. The surface area of all electrodes was approximately 20 x 10^{-6} cm^2.

As seen by scanning electron microscopy, erosion of the Pt30%Ir electrodes was found after all levels of charge injection ranging from 200 to 3200 $\mu C/cm^2 \cdot ph$ for 24 hrs or 1 week (200 QD). The pattern of erosion was that of a peripheral annulus at the lower QD levels with an increase in erosion of the entire facet proportional to increasing levels of QD. To date, pure iridium microelectrodes have shown no erosion following injections of QD up to 3200 for 24 hrs. This remarkable durability of iridium holds great promise as a metal of choice for long-term neural stimulation, where metal dissolution must be avoided.

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266.1 A TWO-ELEMENT OCULOMOTOR PLANT MODEL RESOLVES PROBLEMS INHERENT IN A SINGLE-ELEMENT PLANT MODEL. H. P. Goldstein* and D. A. Robinson (SPON: R. Burde). Washington University, St. Louis, MO 63110, and The Johns Hopkins University, Baltimore, MD 21205.

Unit recording from the abducens nucleus suggests the need to model the oculomotor plant as a cascade of two spring-dashpot elements, with time constants about 20 and 300 msec, instead of a simpler single spring-dashpot element, with a time constant about 200 msec. When analyzed, both models predict nearly indistiguishable inputs for slow eye movements. Both models predict a linear "rate-position" curve as well as a ramp-like increase in neuron firing rate during constant velocity eye movements. Although the predicted inputs during sinusoidal tracking differ, the difference is small at the low frequencies tested physiologically and the disparity might not be apparant from experiments. Only during saccadic eye movements do the two models have disparate inputs. To compare them directly, the inverse of both models were simulated on a digital computer where the input to these inverse-models was eye position and the output was required force generated by the oculomotor muscles. The averaged waveform of ten identical 20° monkey saccades was the input; the movement lasted 50 msec. Both models predicted a force that increased abruptly at the beginning of the saccade. The initial rise ended after 8 msec and before the eye had moved 3°. The force obtained by the single-element model peaked above 120 grams during the first 20 msec of the saccade and decreased smoothly to the post-saccadic level during the last 30 msec of the saccade; the post-saccade force remained constant. In contrast, the required force of the two-element model peaked at only 50 grams, stayed virtually constant during most of the saccade and started to decay 10 msec before the end of the saccade. The force continued decaying exponentially with a 100 msec time constant well after the saccade had ended. The initial fast development of force early in the saccade, the plateau of force at 50 grams during the saccade, and the exponential decay in force after the saccade are all features found in experiments using implanted strain gauges (Collins et.al., 1975). Hence, the two-element model performs physiologically when generating both fast and slow eye movements whereas the single-element model does not. (This work supported by NIH grants EY07047, EY01765, and EY00598.)

266.2 THE VESTIBULO-OCULAR REFLEX IN RABBIT, AS INTERPRETED USING THE MOORE-PENROSE GENERALIZED INVERSE TRANSFORMATION OF INTRINSIC COORDINATES. J.I. Simpson and A. Pellionisz, Dept. Physiol. Biophys. N.Y.U. Med. Ctr., New York 10016.

The vestibulo-ocular reflex (VOR) provides a specific model for a quantitative application of the tensorial treatment of sensorimotor coordination introduced by Pellionisz & Llinas (1979,1980). Their theoretical approach, treating vectorial expressions in intrinsic reference frames, raised two key issues: the non-orthogonality and the potential overcompleteness of these reference frames. These issues can be presented by considering the kinematic analysis of eye movements, although the broader focus is the interpretation of the sequential transformations in the VOR, where the frames may be overcomplete. Eye movement is typically treated as a 3 degree of freedom rigid body rotation caused by the action of 6 muscles, each with its own rotation axis. The excess of the number of muscle rotation axes over the number of degrees of freedom means that, unless constraints are introduced, a particular eyeball angular velocity cannot be resolved into a unique set of contravariant component rotations in the 6 dimensional muscle reference frame. Rather, an infinity of such component sets exists. For the eyeball, this problem can be "resolved" by reducing the dimensionality from 6 to 3 by muscle pairing. This approximation permits a matrix description of the input-output transform, but without interpretation of the network transformations in between. Moreover, such reduction may not be applicable to other motor systems, e.g. the neck. Other approaches exist, but they all involve some approximation. One alternative approach uses the Moore-Penrose generalized inverse, as proposed by Pellionisz (1983). This approximation preserves the eigenvectors of a matrix. From measurements of the physical arrangement of the rabbit's extraocular muscles, the Moore-Penrose generalized inverse has been calculated and is interpreted as the oculomotor metric tensor in the VOR transform. Some advantages of this approach are that it is general, the identity of the individual muscles is preserved, and the interim neuronal transformations can be represented. Keeping the intrinsic coordinates distinguishable is also likely to prove important in extending the analysis of eye movements from kinematics to dynamics, and beyond, to the functional geometry of neuronal control. Supported by NIH grant NS13742 from NINCDS.

266.3 AN ANALYSIS OF COMPONENT-CROSSCOUPLING IN OBLIQUE SACCADES. J.A.M. Van Gisbergen*, A.J. Van Opstal* and J.J.M. Schoenmakers*. (SPON: European Neuroscience Association). Dept. of Medical Physics and Biophysics, Univ. of Nijmegen, 6525 EZ Nijmegen, The Netherlands.

Several recent studies (see King, W. et al., Soc. Neurosci. Abstr., 1983) have revealed that orthogonal components of oblique saccades do not obey the same peak-velocity/duration/amplitude relations as purely horizontal or vertical saccades: The smaller component has a prolonged duration and a reduced peak velocity (stretching). We have made a detailed study of the dynamic properties of horizontal, vertical and oblique saccades in two monkeys. Saccadic eye movements in various directions were measured with our improved version of the double-magnetic induction technique and corrected for the static nonlinearity inherent in this method (Bour, L. et al., IEEE Trans. Bio-Med. Eng.,1984;in press).

We found that onsets of orthogonal components of oblique saccades are so well synchronized in the monkey that a common initiation system seems likely. Saccade vectors obeyed a nonlinear peak-velocity/amplitude relationship in all directions. The peak-velocity/duration/amplitude relationship for components was not fixed, but depended on the relative size of the orthogonal component. For a component with a given size, its duration increased and its peak velocity decreased as the saccade vector to which it contributed turned away from the component direction under consideration. We found that this nonlinear effect (stretching) was negligible for small saccade vectors but became very pronounced in large oblique saccades.

It appears that a model which assumes the existence of synchronized, but dynamically independent pulse generators for horizontal and vertical components must be rejected. An alternative model, featuring a nonlinear vectorial pulse generator followed by a decomposition stage generating component-velocity command signals from the vectorial eye-velocity signal, provides good fit with the data. According to this so-called common-source model, the two nonlinear phenomena observed (curvilinear peak-velocity/amplitude relationship; component stretching in large oblique saccades) are due to a single nonlinearity in the proposed vectorial pulse generator. A possible neural basis for the common-source model, relying partly on data and suggestions from Hepp and Henn (Exp. Brain Res., 52:105, 1983), will be discussed.

266.4 ADAPTIVE NEURAL DYNAMICS OF THE SACCADIC EYE MOVEMENT SYSTEM. S. Grossberg and M. Kuperstein. Center for Adaptive Systems, Boston University, Boston, MA 02215.

Neural circuits for the control of saccadic eye movements are derived from concepts about how saccadic errors are corrected during normal development or after certain adult lesions. These circuits are used to analyse data concerning the role of retina, superior colliculus, peripontine reticular formation, cerebellum, parietal cortex, frontal eye fields, and oculomotor nuclei in eye movement control. The design principles that these circuits instantiate are common to many other sensory-motor systems. We analyse how to calibrate head coordinate maps that transform intended target position commands into agonist-antagonist muscle coordinates using corollary discharge signals; how to compute neural vectors that transform intended target position commands into retinotopic commands that automatically compensate for present position; how to calibrate outflow signals to nonlinear muscles using comparisons with inflow signals; how to attentionally and intentionally modulate movement commands; how to organize and rapidly read-out predictive sequences of pre-planned motions; how to separately calibrate the gains that maintain posture and that control movements; how to explain recovery from lesions of one neural region by adaptive redistribution of processing load to functionally related regions. A functional language is developed with which to describe the new concepts and mechanisms of the theory. Many of these concepts and mechanisms can be used to analyse other sensory-motor systems and to suggest new designs of self-calibrating robots. Applications include explanations of double-flash experiments, flash-electrode experiments, experiments on independent frontal eye field and superior colliculus saccadic control, experiments on visually-guided arm pointing after strabismus surgery, experiments on corrective saccades in the dark, experiments on independent cerebellar control of pulse and step gains, experiments on fractured somatotopy, experiments on inhibition of parietal light-sensitive neurons during saccades, experiments on saccade staircases, experiments on adaptation to curvature-distorting contact lens. Many predictions follow from the explicit nature of the model circuits.

Reference: S. Grossberg and M. Kuperstein, Adaptive neural dynamics of the saccadic eye movement system and general principles of sensory-motor control, 1984.

266.5 A DIFFERENT LOCAL FEEDBACK MODEL OF THE SACCADIC BURST GENERATOR. Charles Scudder. Regional Primate Center and Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195.

D. A. Robinson has proposed a model of the saccadic burst generator which uses a neural replica of "target position in space" for its input, and uses the difference between this signal and an efference copy of current eye position to produce a "motor error" signal which controls the time course of the saccade. The source of the input is not certain, but since the superior colliculus (SC) is strongly implicated in the initiation of saccades, and since it codes desired saccade size (i.e., initial motor error), there have been attempts to incorporate it into the model. If the SC is the source of "motor error," however, it must also code the decline in "motor error" as the saccade progresses. There is no experimental support for this contention. Any lesser role for the SC implies that its output is transformed back into the more rudimentary target position in space signal before it projects to the burst generator.

I propose a modification of Robinson's model which also uses "local" feedback but is able to use the known output of the SC directly. The difference between the excitatory SC input and the inhibitory feedback from the inhibitory burst neurons (IBNs) is integrated mathematically and this "integrated error" is used to drive the pontine excitatory burst neurons (EBNs). As in other models, the EBNs are inhibited by omnipause neurons (OPNs) but the inhibition is weak enough that it can be overcome by the excitatory integrated error to initiate a burst. This process is facilitated by a simultaneous weak inhibitory input to the OPNs which reduces their discharge as the integrated error is growing. Two important consequences of this scheme are that SC projects to long-lead burst neurons (the error-integrator), and that it codes desired saccade size in total (topographically weighted) number of spikes.

Besides making effective use of the SC, this model has other advantages. It accurately reproduces the recorded discharge of the neurons it uses, and it does not need a speculative feedback from tonic neurons. When expanded to a two-dimensional burst generator, the model reduces the velocity and extends the duration of the smaller component of oblique saccades as it must to mimic actual data. Finally, much of the data which supports Robinson's version of local feedback, such as the continuation of the saccade after brief interruption by OPN stimulation, also supports this version.

266.6 ADAPTATION OF VISUAL-VESTIBULAR INTERACTION WITHOUT ALTERATION OF VOR OR VISUOMOTOR PERFORMANCE. M.T.Feran*, R.M.Douglas and G. Melvill Jones, Dept. of Physiology, McGill University, Montreal, Canada H3G 1Y6.

The previously reported ability of cats to improve visual supression of the vestibulo-ocular reflex (VOR) (Douglas et al., Neurosc. Abstr, 8, 269.7) was tested to (1) confirm that the long-term improvement of visual suppression is independent of adaptive attenuation of the VOR, (2) determine the role, if any, of improved full field visuomotor respones (VMR) in the enhanced VOR suppression, and (3) to assess the frequency specificity and velocity dependence of the enhanced suppression.

Eye movements were recorded from 2 alert behaving cats during sinusoidal oscillation of (1) the animal in darkness (VOR), (2) the animal while viewing a striped optokinetic drum mechanically coupled to the turntable (fixed field or FF), and (3) the optokinetic drum rotating relative to the stationary animal. Testing and conditioning sessions were repeated for each cat once weekly over 3 months. Each session consisted of two test periods separated by 2.5 hours of exposure to FF vision during .2 Hz sinusoidal oscillation with a 40 deg./sec peak velocity. VOR, FF, and VMR were tested from .1 to 1.3 Hz (at 40 deg./sec) and from 5 to 80 deg./sec (at 0.2 Hz).

While within each conditioning session the VOR gain consistently declined by 40-50%, it recovered completely after each intervening week of normal cat colony vision. In contrast, the FF gain at the start of each session declined steadily over the 3 months. At the conditioning frequency (.2 Hz, 40 deg./sec), the suppression improved from 9% to 25%. There was less improvement at the higher frequencies. This improved suppression was not due to an improved visuomotor performance since this was unchanged or reduced. Suppression and VMR were always better at lower velocities, but while the FF gain could be predicted from the VOR and VMR gains at the beginning of the experiment, by the end the amount of suppression was much greater than expected.

We conclude that this experimental protocol produced a form of enhanced VOR suppression which could not be attributed to either adaptive attenuation of the VOR or adaptive enhancement of purely visuomotor performance.

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266.7 SELECTIVE EFFECTS OF UNILATERAL EYE PATCHING ON THE VESTIBULO-OCULAR REFLEX AND SACCADES. Erik S. Viirre*, Murari Patodia*, Jon Hore and Tutis Vilis. Dept. Physiol. and Dept. Ophthal., University of Western Ontario, London, Ontario, Canada N6A 5C1.

Visual error signals are necessary for calibration of both the vestibular-ocular reflex (VOR) (Miles et al. Ann. Rev. Neurosci. 4: 273, 1981) and the saccadic system (Optican and Robinson, J. Neurophysiol. 44: 1058, 1980). In the present study we investigated whether selective removal of the visual input from one eye causes an uncalibration in that eye for both the VOR and saccades.

Two Macaca Fascicularis monkeys, trained to fixate visual targets, were subjected to patching of one eye for one week followed by unpatching for one week. This was then repeated for the other eye.

After one week of patching small changes in eye movements were observed in the patched eye. No changes were observed in the unpatched eye. For horizontal saccades these changes in the patched eye were: 1) an increase or decrease in the initial saccade magnitude (range patched eye/unpatched eye = 88 to 115%, 2) development of post-saccadic drift (time constant 50-120 ms, magnitude 1.5 to 4.1 deg in the first 200 ms following the saccade) and 3) appearance of a vertical component in horizontal saccades. In the VOR during horizontal rotation at 0.5 Hz, changes in the patched eye were: 1) a decrease in the gain which was different from that seen for initial saccade magnitude and 2) appearance of a vertical displacement during horizontal rotation that was in the same direction as the vertical component that appeared in the saccadic system. These changes appeared gradually over the patched period and disappeared in the subsequent unpatched period.

In summary removing the visual input from one eye results in selective uncalibration of both VOR and saccadic systems in that eye. Thus a continuous visual input is necessary to maintain calibration. Furthermore the results demonstrate that the saccadic system can be calibrated independently of the VOR and that in these conjugate eye movements, each eye can be calibrated independently.

(Supported by the Medical Research Council of Canada).

266.8 SOME INFLUENCES OF GAZE DEVIATION ON VESTIBULAR NYSTAGMUS AND THE DISPLACEMENT COMPONENT OF THE OCULOGYRAL ILLUSION (OGI). J. N. Evanoff* and J. R. Lackner. Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, Massachusetts 02254.

Alexander found that properties of post-rotary nystagmus are affected by voluntarily maintaining gaze in the direction of the fast phase or slow phase component. Systematic increases in nystagmus frequency with voluntary eye deviation in the fast phase direction and decreases with voluntary gaze in the slow phase direction have become known as "Alexander's Law".

We examined per-rotary influences of gaze deviation on vestibular nystagmus for trapezoidal velocity profiles ($15^{o}/s^{2}$, angular acceleration): gaze deviation in the direction of the fast phase significantly increased slow phase and fast phase amplitude ($p<.01$) and slow phase velocity ($p<.01$); deviation in the slow phase direction decreased all three characteristics, but only marginally. Schlagfeld deviation and beat frequency were unaffected by eye deviation. The ability to alter certain properties of vestibular nystagmus without affecting others provided us with a tool for studying visuo-vestibular-oculomotor interactions.

An observer undergoing angular acceleration and foveating a target light fixed in his median plane will see the target displace from the midline toward the direction of acceleration. This apparent visual displacement is known as the oculogyral illusion (OGI). We have varied the fixation-signal strength necessary during trapezoidal velocity profiles ($15^{o}/s^{2}$) to maintain stable target fixation by using Alexander's principles to increase or decrease reflexive oculomotor activity. Having the target and eyes deviated in the direction of acceleration (also the fast phase direction of the latent nystagmus) increased the magnitude of the OGI ($p<.01$), deviation in the opposite direction decreased it ($p<.01$) relative to the standard condition in which the visual target was in the midline.

We conclude that the OGI involves a central misrepresentation of eye position attributable to monitoring the fixation command necessary to suppress latent vestibular nystagmus. Such monitoring was originally suggested by Whiteside, Graybiel and Niven (Brain, 88:193, 1965). Our ability to modulate the OGI by varying properties of the latent nystagmus provides additional support for the importance of the fixation command in the assignment of visual direction.

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266.9 COMPARISON OF SACCADES EVOKED BY MICROSTIMULATION IN THE FRONTAL DORSOMEDIAL AND PREARCUATE CORTEX OF MONKEY. M. Schlag-Rey and J. Schlag. Dept. of Anatomy and BRI, UCLA, Los Angeles, CA 90024.

Microstimulation was performed to compare eye movements evoked from two areas of the frontal cortex in monkey: the prearcuate frontal eye field (FEF) and dorsomedial cortex (DM). Three monkeys (macaca nemestrina), head fixed, served in this study, one in alternating explorations of FEF and DM. Eye movements were recorded with a magnetic search coil. Other movements were carefully observed but not recorded. Stimulus trains were 10-40 cathodal pulses, 0.2 ms duration, 250 Hz, delivered by a constant current stimulator through tungsten electrodes. Microstimulation was applied at sites where single units were isolated and often the same unit could still be recorded afterward. Reconstruction of the tracks was based on 50 u sections cut in the plane of the electrodes and stained with thionin and with the Merker silver stain. (J. Neurosci. Meth., 9:235-241, 1983).

In both FEF and DM, sites for lowest threshold stimulation eliciting contraversive saccades corresponded to sites where saccade-related activity had been recorded, usually in lower cortical layers. Lowest threshold sites could be several mm apart, for instance in depth of arcuate sulcus and surface of prearcuate cortex. Nevertheless, the 2 foci centered in FEF and DM were clearly separated. Thresholds were lower in FEF (usually 10-20 μA, minimum 4 μA) than in DM (usually 20-50 μA, minimum 6 μA). The minimum latency of evoked saccades was comparable at both places (around 30 ms). Evoked saccades were of two types: 1) either specific vector (constant amplitude and identical direction whatever the initial position of the eye in orbit) or 2) converging to a given terminal location in orbit. Both types could be observed in FEF and DM, even with low currents, but the second type was less frequent in FEF. Both types could be encountered in succession along the same track. A topographical organization of saccade direction was found in DM with upward direction at the top, contraversive in between and downward in the lower part of the mesial face. The rostrocaudal coordinates corresponded to Woolsey's map of the supplementary motor area but the excitable region was the mesial face rather than the upper bank of the cingulate sulcus. Whereas only saccades were evoked rostrally in DM, ear and shoulder movements accompanied saccades evoked from more caudal sites. This study indicates that FEF and DM are probably separate eye fields and that DM may be the oculomotor part of the supplementary motor area. (Grant EY 02305)

266.10 COMPARISON OF SACCADIC EYE MOVEMENTS IN MAN AND MACAQUE. J.S. Baizer and D.B. Bender*, Division of Neurobiology, Department of Physiology, School of Med., University at Buffalo, SUNY, Buffalo, NY 14226.

We compared latency, accuracy and velocity of saccadic eye movements of man and macaque on single- and double-step paradigms. Cynomologous monkeys were trained to press an illuminated key to turn on a central fixation point (FP), to make a 12°, 24° or 36° horizontal saccade, or a 12° vertical saccade to a target LED, and to respond to dimming of that LED by pressing a second key. Humans performed the same task in the same apparatus. Correct responses were rewarded with water (monkeys) or $.01 (humans). On single-step trials, target onset was synchronous with FP offset; on single-step overlap trials, the FP remained on. On double-step trials, a 24° target appeared briefly (30-180 msec), and was followed by onset of a 12° target. Eye movements were recorded with implanted (monkeys) or paste-on (humans) EOG electrodes. The data are based on approximately 14,000 trials per subject.

On single-step trials, the oculomotor system of macaques is faster and more accurate than that of humans. For monkeys, average saccade latency typically ranged from 150 to 230 ms; for humans, average latency ranged from 200 to 280 ms. For monkeys, mean velocity of 12° to 36° horizontal saccades ranged from 450 to 750 deg/sec; for humans, velocity ranged from 250 to 350 deg/sec. Monkeys consistently acquired the target with a single saccade; humans consistently undershot the 24° and 36° targets and required a corrective saccade. On single-step overlap trials, monkeys showed increased variability in saccade latency, but no change in saccade accuracy; humans showed no change in latency but did show increased accuracy, with reduced undershooting.

On double-step trials, for both humans and monkeys, the amplitude of the primary saccade varied with the delay between the second target jump and saccade onset as in the "amplitude transition functions" (ATF's) of Becker and Jürgens (Vis. Res., 1979, 19: 967). The ATF's were generally similar for the two species; humans and monkeys both had modification times in the range of 30-70 ms. However, at delays of 100-200 msec, the amplitude of human, but not monkey, primary saccades fell below 12°. The data suggest that for monkeys, as well as for humans, saccades can be altered by new visual information arriving as late as 30 msec before saccade onset. Supported by NEI grants EY02245 and EY02230.

266.11 POWER SPECTRAL ANALYSIS OF HUMAN SACCADES. C. M. Harris*, J. Wallman and C. A. Scudder (SPON: M. D. Gottlieb). Dept. of Biology, City College, City Univ. of N.Y., New York, NY 10031 and Dept. of Physiol. and Biophysics, Univ. Washington, Seattle, WA 98195.

Human saccades, recorded using the search coil technique, were individually analysed by an exact digital Fourier transform. This algorithm overcomes the shortcomings of Fast Fourier Transforms and can accurately measure power spectral components down to -60 dB without artifacts. As expected, a close correspondence between the power spectra and amplitudes of saccades was found. Surprisingly, at frequencies above 15 Hz, the power spectra of saccades exhibited unusual features. Although these features contribute very little to the total energy in a saccade, they seem to reflect an underlying structure not normally discernible from usual eye movement records.

In particular, we consistently find a local minimum, usually sharply defined, at a frequency whose reciprocal approximates the saccade's duration. (Other maxima and minima were always evident at higher frequencies but were less readily categorised because of noise.) Monotonic relationships were found between the reciprocal frequency of this local minimum and the amplitude, duration, and peak velocity of saccades, suggesting that the minimum is a measure of some basic saccadic parameter. We hypothesise that the frequency of this local minimum is related to the width of the burst component of the motoneuron discharge during saccades. A comparison of this parameter with the activity of monkey ocular motoneurons during saccades will be presented.

266.12 CONCURRENT EFFECT OF INTRAVENOUS BENZODIAZEPINE ON SACCADIC EYE VELOCITY AND SEDATION. V. Matsuo, G.A. Chrousos* and D.W. Hommer. NEI, Clinical Branch, and NIMH, Clinical Neuroscience Branch, NIH, Bethesda, MD 20205.

Previous studies have shown that benzodiazepines (BZ) lower saccadic eye velocities without affecting accuracy or latency. The purpose of this study was to establish a dose-response curve as a measure of BZ receptor sensitivity, using saccadic eye velocity and sedation as indices.

Ten healthy normal volunteers were used (7 females, 3 males; age range 25-36 yrs). They gave informed consent. Diazepam or saline placebo was administered single blind in doses of 4.4, 4.4, 8.8, 17.5, 35 and 70 μg/kg at 15 minute intervals. Subjects performed a saccadic tracking task within 2-5 minutes of receiving each dose. They viewed a horizontal array of 5 LED's that were placed centrally, and 7.5° and 15° to the right and left. The LED's were activated in a quasi-random order and the subject was instructed to move his eyes to the target as quickly and accurately as possible. Eye position was monitored using IR scleral reflection. Target position, eye position and eye velocity were displayed on a chart recorder and stored on FM tape for later analysis. Total system bandwidth was 100 Hz.

Saccadic velocity showed a dose-dependent decrease beginning with a cumulative dose of 35 μg/kg. An unexpected finding was that saccades greater than 6° were most severely reduced. After the 6th dose the velocity of 15-30° saccades was reduced by as much as half that of the control saccades. Smaller saccades were also affected, but to a lesser extent. At the highest dose levels subjects tended to make few large saccades, but rather broke the attempted movement into several 5-6° saccades.

Subjects rated their level of sedation on an analog line scale after each dose. Self-rated sedation increased in a significant dose-dependent fashion. An unexpected finding was that, as with saccadic velocities, self-rated sedation increased significantly only after 35 μg/kg cumulative dose. Further, the decline in alertness closely paralled the decline in velocity of large saccades (pooled correlation, $r = 0.82$, $p<0.01$). This suggests that both the decrease in eye velocity and the sedation associated with diazepam administration are mediated through BZ receptors and that they may prove to be a measure of BZ sensitivity in humans.

266.13 HUMAN EXPRESS-SACCADES: GOAL DIRECTED EYE MOVEMENTS AFTER EXTREMELY SHORT REACTION TIMES
B. Fischer and E. Ramsperger*. Dept. Neurophysiology, Univ. Freiburg, D-7800 Freiburg.

Subjects viewed a central fixation point (0.25 deg) on a dim background (0.1 cd/m^2) with their heads stabilized by a chin rest. Horizontal eye movements were monitored by a simple infrared light technique with a resolution of 0.2 deg. They were asked to saccade to a peripherally (4 or 8 deg to the right or left) occurring target (0.25 deg) upon its onset. Saccadic reaction times (SRT) were determined by an electronic threshold detector.

If - after 2 s - the fixation point was extinguished some time (e.g. 200 ms) before target onset the distribution of the SRT's was clearly bimodal with one narrow peak around 100 ms (express-saccades) and a broader peak around 150 ms (regular saccades). Control measurements, where the time of fixation point offset, the location of the target, or the time of target onset, were randomized, ensured that the early peak was indeed due to saccades visually triggered by the onset of the target. The proportion of express-saccades increased (up to 80 %) and the stability of their reaction times decreased (down to ± 6 ms) by daily practice.

If the fixation point remained visible throughout the trial mean SRT's were above 200 ms with large (± 30 ms) standard deviations. Under this condition express-saccades occurred only at a very low rate (≤ 10 %) and were wrong in amplitude by more than 20 %.

These observations parallel those reported for the monkey (Fischer, B. and Boch, R., Brain Res., 260: 21-26, 1983). They show, that without active fixation the visual-to-oculomotor system is in a state of readiness to execute the next saccade correctly after about 100 ms from target onset.

266.14 MIDFLIGHT ARREST OF AN AVIAN SACCADIC COMPONENT BY VISUAL MOTION.
J.C. Letelier* and J. Wallman. Dept. of Biology, City College, City Univ. of N.Y., New York, N.Y. 10031.

Saccadic eye movements, once initiated, generally are completed without reference to visual events. In view of the rapidity of mammalian saccades and the relatively slow retinal processing of visual information, this finding is hardly surprising.

In many birds, each saccade exhibits a prominent oscillation of the eye. In eye movement records, the duration (approximately 200 msec) of this oscillation defines the beginning and end of saccades. The oscillation is mostly cyclorotational and approximately sinusoidal with an amplitude of several degrees and a frequency of 20-30 Hz. The mechanism by which this oscillation is generated is quite unknown. One possibility is that the oscillation is an integral part of the saccade and is therefore produced by the saccadic burst-generating machinery. Alternatively the oscillation may be superimposed on the output of the saccadic generator, rather than being an essential part of it. We report here that this saccadic oscillation can be prematurely terminated by presenting large-field visual motion during the saccade.

Eye movements were recorded by the search coil/magnetic field method from young white leghorn chickens, placed within a striped cylinder. Eye movement signals were differentiated and went to logic circuitry that detected saccades by requiring a period of low eye velocity to be followed by a period of high eye velocity of appropriate duration. On approximately every other saccade, the onset of the saccade triggered a brief (100-200 msec) rotation of the surrounding cylinder.

The results were that the presence of the visual motion caused the duration of the saccade, as measured by the duration of the oscillation, to be dramatically shortened. The latency of this effect from the start of cylinder rotation was approximately 50 msec. We do not know at this point whether it was only the saccadic oscillation that was quenched by the presence of visual motion or whether the saccadic trajectory as well was truncated. In either event, it seems remarkable that, while travelling at hundreds of degrees per second, the eye is able to respond to brief episodes of low-velocity retinal image slip.

266.15 EFFECTS OF UNILATERAL CEREBRAL CORTEX LESIONS ON EYE MOVEMENTS IN MONKEYS.
R.J. Tusa, D.S. Zee and S.J. Herdman, Johns Hopkins University, Baltimore, MD. 21205

Cerebral cortical lesions are known to impair ipsilateral pursuit and may impair contralateral saccades, but this has not been quantified after an acute lesion. To better understand the role of cerebral cortex on eye movements, we made unilateral lesions in two trained rhesus monkeys. Eye movements were recorded (search coil) before and during the first 3 days after aspiration of all neocortex in one hemisphere except for mesial temporal and orbital frontal areas.

Vestibulo-Ocular Reflex (VOR): Despite an eye deviation toward the lesioned side, VOR quick and slow phases crossed the midline. For 60-300°/s steps in darkness, the VOR gain and time constant were within 10% of pre-op values.

Visual Tracking: Tracking of a 0.5 deg light moving toward the side of the lesion elicited catch-up saccades but no smooth pursuit. Tracking away from the lesioned side elicited normal smooth pursuit up to 15°/s where eye velocity saturated (max pre-op > 120°/s) but no catch-up saccades were generated. Tracking stopped as the target crossed the midline even though the target stayed within the intact visual field. Targets moving vertically elicited catch-up saccades but smooth pursuit was limited to 1°/s (max pre-op > 80°/s).

Optokinetic Nystagmus (OKN): For 5-300°/s steps, the initial "pursuit" component was less than 5°/s toward and 20°/s away from the lesioned side (max pre-op > 60°/s). For stimuli away from the lesioned side there was a normal slow build up of OKN and normal afternystagmus (OKAN). For stimuli toward the lesioned side the slow build up and OKAN were never greater than 5°/s (max pre-op > 60°/s).

Rapid Eye Movements: The amplitude of VOR and OKN quick phases directed toward the lesion were normal; those away from the lesion were decreased (up to 50%). Spontaneous saccades were made in all directions. Target-triggered saccades were 20% hypermetric toward the lesioned side and not made away from the lesioned side. Vertical saccades had a 20% horizontal component toward the lesioned side. Velocities of all quick phases and saccades were only slightly reduced.

Conclusions: Although the eye deviated toward the lesioned side, the eye crossed the midline with both slow and quick phases generated by the VOR. Smooth pursuit was deficient in all directions. There was a deficit in the "pursuit" component of OKN in both horizontal directions. There was also a deficit in the OKN input into the OKAN mechanism (velocity storage system) for drum rotations toward the lesioned side.

266.16 THE THRESHOLD SPECTRAL SENSITIVITY OF AN EYE MOVEMENT IN DAPHNIA MAGNA.
T.R. Consi and E.R. Macagno. Columbia University, New York, NY 10027.

The single compound eye of the small freshwater crustacean Daphnia magna exhibits a variety of eye movements, including tremor, saccades and optokinetic nystagmus (Frost, J. exp. Biol. 62:175-187, 1975). The recent determination that the compound eye contains at least three spectral classes of photoreceptors (peak sensitivities at about 450, 510, and 590nm; Schehr and Macagno, Neuroscience Abstracts 9: 325, 1983) prompted us to examine in detail the wavelength dependence of one of these light-evoked responses, the ventral rotation of the eye in response to a flash of light anywhere in the visual field, an optomotor behavior we have named eye flick.

The stimuli were presented to Daphnia held in place on the axis of a conical screen. The full screen subtended 180° dorso-ventrally and 60° left-to-right at the eye, whose axis was aligned with the center of the screen. For each wavelength, the threshold for eye flick was determined and the relative sensitivity (using the sensitivity to 517nm light as the standard) was calculated.

Three stimulus conditions were used: (1) full-field, in which the whole 180°x60° screen was illuminated nearly uniformly; (2) dorsal, in which a 30°x60° region at the dorsal margin of the screen was illuminated; and (3) ventral, same as (2) but at the ventral margin. The full-field spectral sensitivity curve shows a maximum at about 530nm, almost as high a sensitivity at 365-400nm, a local minimum (1 log unit below maximum) at 420nm, and a shoulder at about 600nm. The dorsal sensitivity shows an overall maximum at 400nm, local maxima at 440nm and 517nm, and a decrease of approximately 0.75 log unit with respect to the full-field relative sensitivity at 600nm. The ventral sensitivity is very similar to the full-field sensitivity, except at wavelengths longer than 570nm, where it is about 0.5 log unit less.

A hypothesis that could explain the full-field spectral sensitivity results is that the middle (510nm) and long (590nm) wavelength receptors provide positive inputs to the eye flick system, whereas the short (450nm) wavelength receptors inhibit this response. In contrast, the dorsal sensitivity results suggest that the middle and short wavelength photoreceptors provide positive inputs and the long wavelength receptors are not involved in producing the eye movement. Efforts are currently under way to test these hypotheses. (Supported in part by NIH Grant NS 14946.)

266.17 CONTROL OF HEAD POSITION IN THE LIZARD, AMPHIBOLURUS BARBATUS, TO ROTATIONAL MOVEMENTS AROUND THE BODY AXES.
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Many lizards tend to stabilize their retinal image by responding with compensatory head and/or eye movements to rotational movements around the eye axes. Thus, eye positions relative to the surround remain unchanged, i.e. 'fixed in space'. Control of eye position was studied in bearded dragons, Amphibolurus, by immobilizing the animals without eye or head motion. Animals were exposed to sinusoidal motion around the body long axis (roll), around a vertical axis through the center between both eyes (torque) and around a transverse axis through both eyes (pitch response). The change of angular eye/head position to such stimuli was recorded with a video camera followed by a frame-by-frame analysis (40 ms and 200 ms time resolution) of angular head and eye position as a function of body position, extent and velocity of angular body motion.

Amphibolurus responds to rotational motion around the body long axis with a change of angular position of the head keeping the eye position constant with respect to the head. For small angular oscillations (< 25 deg peak-to-peak) the head hardly follows body motions (less than 4 deg), whereas oscillations between 25 and 60 deg induce angular head motions approximating ratios of 1:2 to 1:6 between head and body angle (measured relative to the horizontal plane). For angular extent of oscillations exceeding 60 deg, head and body motions are correlated 1:1. Similar response features were found for rotational motion around transverse and vertical axes defined above.

In contrast, preliminary experiments conducted with the water dragon, Physignathus lesueurii, show similar responses to rotational motion around the vertical and transverse axes. However, head positions are correlated almost 1:1 to body motions upon rotational motion around the body long axis; thus, eye motions display the features described for head motions in Amphibolurus.

Supported by a Heisenberg stipend (Ec 56/5-2) by the 'German Research Foundation' (Deutsche Forschungsgemeinschaft).

DEVELOPMENT AND PLASTICITY: SPINAL CORD, MOTOR NEURONS, AND MUSCLES

267.1 POSTNATAL DEVELOPMENT OF RAPID LIMB MOVEMENTS IN NORMAL AND SPINAL KITTENS. N.S. Bradley and J.L. Smith. Dept. Kinesiology, UCLA, CA 90024.

When a cat gets water on its paw or objects lodged in it, a paw-shake response (PSR) rids the paw of the stimulus by repetitive and rapid alternation of flexion and extension of the entire limb (Smith, et al. J. Neurophysiol. 43:1980). The purpose of this study was to characterize development of the PSR in normal and spinal kittens.

To determine onset of the PSR, 13 normal kittens and 10 kittens spinalized (T12) at 14 days of age were tested daily from birth until the PSR was reliably elicited. Onset of the PSR was credited if at least one cycle of rapid flexion and extension occurred in response to tape or water applied to the paw. Onset occurred within 21-33 days of age in normal kittens and not before they were able to maintain stance during the postural perturbation. Onset of the PSR in spinal kittens occurred within 1-3 days following spinalization, preceding onset in normal littermates by 1-2 wks but was elicited infrequently. Early PSRs were 1-2 cycles in all animals.

At 6 wks of age the lateral gastrocnemius (LG), tibialis anterior (TA) and vastus lateralis (VL) were implanted in 2 normal and 2 spinal kittens to determine cycle characteristics and intralimb synergies. EMG records indicated PSRs ranged 1-9 cycles in normals and 1-7 cycles in spinal kittens. Both groups averaged 3 cycles per PSR with cycle periods (the interval between LG burst onsets) of 92-128 ms and LG burst durations of 22-40 ms. Average onset for the TA ranged 61-76% of cycle with burst durations of 26-72 ms across kittens. Average onset for the VL was 44-66% of cycle with burst durations of 16-46 ms. No differences were found between groups. However at this age, the PSRs of kittens differ from those of adults in that they have fewer cycles (normal adults have 8-9 cycles and spinal adults have 11-12 cycles), and kittens have longer cycle periods (normal and spinal adults have cycle periods of 83-90 ms).

These data suggest that the necessary circuitry for the PSR is present in the spinal cord 1-2 weeks before onset in normal kittens and may be inhibited by rostral centers until postural control is sufficient to accomodate the response. However, at 6 wks of age the PSR in normal and spinal kittens is similar suggesting that spinalization can hasten the onset, but not the maturation of the response.
Supported by NS 19864.

267.2 POSTNATAL GROWTH OF MEDIAL GASTROCNEMIUS MOTONEURONS IN THE CAT. W.G. Tatton, M. Hay* and I.C. Bruce. Playfair Unit, Univ. of Toronto, Toronto, Ont. M5T 2S8 Canada.

Cat muscle nerve axons (Nystrom B., Acta Neurol. Scand. 44: 265-294, 1968) show a unimodal diameter distribution of 1-5 μm at birth, followed, at about 20 postnatal days (PD), by a sudden change to a bimodal distribution of 1-3 and 4-7 μm, resembling the adult. This appears to parallel somal growth in unidentified ventral horn neurons. Retrograde transport of horseradish peroxidase (HRP) (Sato et al., J. Comp. Neurol. 175: 27-36, 1977) showed a bimodal motoneuron (MN) volume distribution at birth (2-4 and 6-24 x 10^3 μm^3), suggesting a dissociation of somal and axon growth. In addition, recent work has shown that the MNs' dendrites attain adult calibre distributions only after 60PD.

In the present study, 5% HRP was selectively injected into medial gastrocnemius (MG). Filled MNs were measured by superimposing two circles, one just within (min), and the second completely surrounding (max) the irregular MN soma cut through the nucleolus. Two-dimensional (2-D) distributions relating min. and max. clearly differentiate two adult size subpopulations. 26 MG pools were examined in cats aged 3PD (69 gestational days) to adulthood, 12 in the 15-26PD period. The 2-D distributions revealed three somal growth phases: 1. slow growth from 3 to 19PD (MN somata small, uniform of size and distributed unimodally); 2. rapid size differentiation between 19 and 24PD (bimodally distributed with appearance of larger MNs); 3. subsequent slow phase from 24PD to the adult distribution. Serial section 3-D reconstructions revealed a narrow MN string within L7-S1 at all ages. During 19-24PD the rapidly growing MNs were randomly distributed through the pools with no rostrocaudal predilection. Application of Sato et al. formula to our MNs also showed a unimodal distribution for somal volumes at birth, and a bimodal one in adults.

Our findings indicate that MG MN somata and their axons are coordinated in their development and establish that somal size differentiation is complete (26PD) well before attainment of the adult dendritic configuration (after 60PD). Further, the 19-24PD growth burst reflects the differentiation of a large MN subpopulation. The two subpopulations may represent alpha and gamma MNs, and will be considered in relation to the postnatal development of spindle efferent innervation and the appearance of fast and slow muscle properties.
Supported by MRC of Canada Grant MA5218.

267.3 LECTIN HISTOCHEMISTRY OF IN VITRO MYOGENESIS OF NORMAL AND DYSTROPHIC CHICK EMBRYO PECTORAL MUSCLE PRIMARY CULTURES. E.L. Hogan, B.A. Schulte* and K.C. Leskawa* (SPON: G.E. Landreth). Departments of Neurology and Pathology, The Medical University of South Carolina, Charleston, SC 29425.

Complex carbohydrates of cultured normal and dystrophic chick embryo muscle cells were analyzed by lectin histochemical techniques, during various stages of myogenesis in vitro. By the end of the myogenic process, over 70% of the nuclei were within mutinucleated structures. An increase in creatine kinase activity paralleled myotubule formation. Paraffin sections of cultures at each myogenic phase were stained with twelve different lectin-horseradish peroxidase conjugates, some with prior enzymatic treatment.

No significant differences were noted between normal and dystrophic muscle cells, at any myogenic stage. In all cultures, a lack of terminal α- or β-galactose, α- or β-galactosamine and α-fucose was observed, evidenced by the absence of staining by several lectin-HRP conjugates. These observations indicate that dystrophic muscle cells in culture do not biosynthesize a unique glycopeptide containing these structures.

The absence of terminal galactose, in addition to an intense staining with alcian blue and the observation that peanut lectin binds only after exogenous sialidase treatment, suggests (1) an abundance of sialic acid residues and (2) the absence of an intrinsic, membrane-associated ecto-sialidase activity. Staining of sialic acid residues was heavy and linear on the plasma membrane with numerous small clusters plus some intracellular granules. Staining with LFA lectin (specific for sialic acid) substantiated these findings.

An abundance of α-mannose residues was shown by Con A binding, which was strong and uniform (i.e., no clusters) on the cell surface, at all stages of differentiation.

The staining pattern of GSA II lectin (α- or β-GlcNAc) changed during myogenesis, from being fairly light and even in myoblasts to being heavier and concentrated in focal patches near the surface on differentiated myotubules. Abolition of staining by salivary amylase or malt diastase suggested redistribution of intracelluar glycogen.

The binding of LCA lectin (specific for biantennary oligosaccharides) progressed from a light, granular pattern in the cytoplasm of single cells to being much greater and more restricted to the surface of myotubules.

Supported by the Muscular Dystrophy Association.

267.4 DEFICITS IN MUSCLE AFFERENT FUNCTION FOLLOWING REINNERVATION. J.B. Munson, W.F. Collins, III, and L.M. Mendell. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY, 11794.

We examined peripheral and central actions of muscle afferents 3 to 9 months following section and resuture of the MG muscle nerve. Single afferents were impaled in the dorsal root (Honig, et al., J. Neurophysiol. 49) and their receptors characterized as "parallel" or "series" using muscle stretch and contraction. Electrical stimulation of the afferent and/or spike-triggered averaging were used to test for its monosynaptic projection to the motoneuron pool. This was evidenced by recording an extracellular field potential (focal synaptic potential: FSP; Munson et al. J. Physiol. 296) with 3M NaCl micropipettes left in place in the MG motoneuron pool. Data were obtained from 7 normal and 10 experimental cats:

UNCUT AFFERENTS (n=70)

	Parallel	Series	Unphysiol.	Inexcit.	Total
FSP Recorded	40%	--	--	--	40%
No FSP Recorded	20%	37%	3%	--	60%
TOTAL	60%	37%	3%	--	100%

REGENERATED AFFERENTS (n=123)

	Parallel	Series	Unphysiol.	Inexcit.	Total
FSP Recorded	25%	1%	7%	3%	36%
No FSP Recorded	32%	7%	16%	9%	64%
TOTAL	57%	8%	23%	12%	100%

In both populations, about 60% of afferents show "in parallel" (presumably muscle spindle) responses and about 40% of afferents generate FSPs. Striking differences include (i) few regenerated afferents show "in series" (presumably tendon organ) responses, (ii) many regenerated afferents are either inexcitable (12%) or require excessive muscle stretch or probing (23%) for their excitation and (iii) many "in parallel" regenerated afferents fail to generate an FSP. This could result from reduced synaptic efficacy following injury (Gallego, et al., J. Physiol. 306) or from muscle spindles being innervated by Ib afferents. Note that one regenerated afferent producing an FSP (a presumed Ia) showed "in series" (presumed tendon organ) behavior. In both populations the fastest "in parallel" afferents usually generated FSPs, suggesting that the largest Ia afferents successfully reinnervated muscle spindles. We conclude that sensory reinnervation of muscle receptors is strongly biased toward spindles and that even after 9 months many muscle afferents (30%) have not re-established functional contact with muscle. Supported by NIH Grants NS15913 (JBM), NS06407 and NS 20264 (WFC) and NS14899 and NS16996 (LMM).

267.5 ACTION POTENTIALS PRODUCED BY DENERVATION OF TONIC FIBERS IN RAT EXTRAOCULAR MUSCLES. A.Y.Bondi, J.Jacoby and D.J. Chiarandini. Depts. of Ophthal. and Physiol. and Biophysics, New York University Medical Center, New York, N.Y. 10016

Mammalian extraocular muscles comprise various populations of twitch or singly innervated fibers (SIFs) and multiply innervated fibers (MIFs). Normally, SIFs can generate action potentials (APs) while one type of MIFs, the tonic fibers of the global or inner layer, produce small, graded, Na-dependent responses or slow peak potentials. Moreover, in normally innervated muscles, MIFs do not respond to anodal break stimulation while SIFs infrequently fire APs. Rat inferior rectus muscles were denervated by section of the inferior rectus branch of the oculomotor nerve. Following various survival periods, the muscles were dissected and recordings were made from the global layer in a chamber perfused with oxygenated saline containing 10 mM Ca. Resting potentials ranged from -30 to -68 mV but fibers were held at -80 mV during recording. In our initial experiments with denervated muscles, the identity of MIFs was confirmed morphologically by tracing fibers which had been labeled with Lucifer yellow following recording. Since denervated MIFs retained the characteristically long membrane time constant (τ) and high input resistance in comparison with SIFs, in subsequent experiments τ was used for fiber identification.

After denervation the excitability of tonic fibers changed. We found that following 6-9 days of denervation, global MIFs or tonic fibers produced APs by both depolarization and anode break stimulation. In some instances an anode break AP could be elicited with a hyperpolarizing pulse as low as 20 mV. When $[Na]_o$ was reduced, the dV/dt max of the AP of MIFs was either reduced (20% Na) or eliminated (0 Na). 10^{-6} M Saxitoxin had a more potent depressant effect on dV/dt max of the AP of MIFs than 10^{-5} M TTX, although neither toxin completely abolished the response. Preliminary data demonstrate that three weeks after denervation, reinnervation is present as indicated by the existence of evoked endplate potentials. At this time most MIFs retain the capacity to produce APs although all have mepps and some produce epps. Supported by USPHS grants EY01297 and EY07009.

267.6 MOTOR UNIT PROPERTIES FOLLOWING CROSS-REINNERVATION BY CAT MEDIAL GASTROCNEMIUS MOTONEURONS. R.C. Foehring*, G.W. Sypert, and J.B. Munson (SPON: W.A. Friedman). Dept. of Neuroscience, Univ. of Fla. Coll. of Medicine, Gainesville, FL, 32610.

Acute experiments using intracellular techniques were performed 8-10 months following cross-reinnervation of lateral gastrocnemius (LG) and soleus (Sol) muscles by the medial gastrocnemius (MG) nerve. The following data were obtained from 14 normal (Fleshman et al., J. Neurophys. 46), four self-reinnervated (Foehring et al., Neurosci. Abs. 9), and six cross-reinnervated cats:

	FF	FI	FR	S
Normal (148 cells)	45%	6%	26%	23%
Self-reinnervated (68)	44%	4%	21%	25%
X-LG (51)	53%	4%	25%	18%
X-Sol (28)	7%	7%	21%	64%

Following self-reinnervation the normal distribution of motor unit types is reestablished. X-LG shows close to a normal distribution, with perhaps a slight bias towards fast units. X-Sol shows a strong bias towards slow units (normal Sol contains virtually all S units). Since the X-LG distribution is near normal and 60% of the MG motoneurons (MNs) innervated LG muscle in these experiments (33% in Sol, 7% non-connected), it is unlikely that the distribution in Sol can be explained by selective reinnervation. More likely, MG MNs are incompletely converting Sol muscle fibers. In addition MG MN properties may be influenced by the muscle innervated. Population means for MN properties are (± SEM):

	Axonal Conduction Vel.	Rheobase (nA)	Input (Mohm) Resistance	AHP Half Decay Time
Normal	96±1 m/s	16±1	0.9±0.1	22±1 ms
Self-re	90±2 m/s	15±1	1.0±0.1	27±1 ms
X-LG	96±2 m/s	15±1	1.3±0.1	24±1 ms
X-Sol	89±2 m/s	9±1	1.9±0.2	37±3 ms

Input resistance is elevated in X-re MNs, especially in the X-Sol. X-LG MNs have properties similar to normal MG MNs suggesting that X-LG MNs represent a random sample of MG MNs. X-Sol MNs have significantly lower axonal C.V. and rheobase, and longer AHP half-decay time than normal. These collective data are suggestive that some MG MNs fail to convert Sol muscle fibers, and that MN properties may be influenced by the muscle innervated. Supported by NIH grant NS15913, the MRS and RERDS of the VA, and the W.L. Gore Co.

267.7 FIRING PATTERNS AND RECRUITMENT BEHAVIOR OF SINGLE EMBRYONIC MOTONEURONS. M.J. O'Donovan. Department of Physiology and Biophysics, The University of Iowa, Iowa City, IA 52242.

The isolated spinal cord of the chick embryo is capable of generating spontaneous episodes of rhythmic motor activity when maintained in-vitro (Landmesser and O'Donovan, J. Physiol. 347:189, 1984). The goal of the present investigation was to establish the firing patterns of single motoneurons during spontaneous activity. For this purpose the deafferented, lumbosacral spinal cord was removed from St.36 embryos and superfused with oxygenated Tyrodes at 30°C. The discharge of single motoneurons was recorded extracellularly using tungsten or glass microelectrodes positioned within the lateral motor column. Motoneurons were identified by antidromic invasion and spike-triggered averaging of the orthodromically propogated muscle nerve spike. During spontaneous activity motoneurons were recruited when a threshold level of neural activity was generated within the parent motoneuron pool. Motoneurons belonging to the same pool exhibited a range of recruitment thresholds. Once recruited, unit firing rate was modulated in a manner paralleling the variation in summed activity of the parent motoneuron pool. These results suggest: 1) An orderly pattern of motoneuron recruitment is established early (St.36) in development of the chick embryo. 2) As a result of variations in recruitment threshold, motoneurons innervating the same muscle may be subjected to different activation patterns (eg. aggregate number of impulses) during their development.

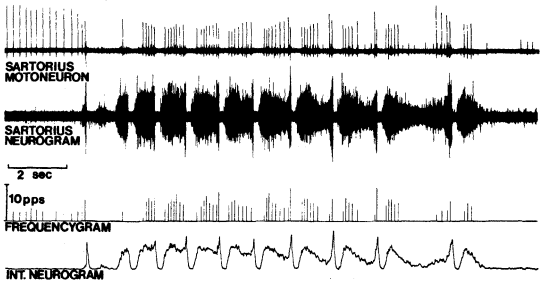

Fig. 1. Firing pattern of a sartorius motoneuron (Cond. velocity=1.3m/s) during a spontaneous burst sequence. Note the pronounced frequency dependence of the spike amplitude.

267.8 AXOSOMATIC INNERVATION IN THE MOTOR TRIGEMINAL NUCLEUS OF THE NEONATAL RAT. L.M. Hemmendinger & V.S. Caviness, Jr. E.K. Shriver Ctr. & Mass. Gen. Hosp., Boston, Ma. 02114

Adult rat trigeminal motoneurons receive axosomatic innervation from 4 morphologically distinct terminal classes. Terminals may contain spherical (S) or pleomorphic (P) synaptic vesicles and either electron lucent (L) or dense (D) axoplasm (Card & Moore, 1979). The characteristic frequency of innervation by each population may emerge via competition between classes for available postsynaptic sites on the motoneuron surface and/or by programmed ingrowth of specific terminal classes over time. As a necessary prerequisite for distinguishing between these 2 possibilities, we compared the terminal morphologies and distribution frequencies of axosomatic contacts present in the neonatal rat motor trigeminal nucleus (MTN) with those in the adult MTN. Adult data is from Hemmendinger & Moore (1983).

On P1, trigeminal motoneurons receive an average of 15 axosomatic contacts, compared with 51 axosomatic contacts to adult motoneurons. Thus, less than a third of the axosomatic innervation to the trigeminal motoneurons appears to be established on P1. This innervation is formed by axonal terminals which resemble 3 of the 4 morphologically distinct terminal types present in the adult MTN. The percentage of each morphologically distinct axosomatic terminal type in the P1 MTN (mean ± std. dev.) is compared below with the percentage of each terminal population present in adults.

	S,L	S,D	P,L	P,D
P1 (15 cells)	57 ± 16	14 ± 8	28 ± 16	0
Adult (20 cells)	30 ± 4	11 ± 3	26 ± 6	34 ± 7

In contrast to the adult, the predominant vesicle morphology in axosomatic terminals at P1 is spherical, and a larger proportion of the terminals contain lucent axoplasm. Terminals containing pleomorphic vesicles and dense axoplasm appear to be the last to form axosomatic connections with the trigeminal motoneurons. Future work will focus on defining the time course of innervation of motoneurons contained in different functional pools within the MTN.

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267.9 AN IN VITRO PREPARATION FOR THE STUDY OF FORE- AND HINDLIMB COORDINATION DURING DEVELOPMENT OF THE BULLFROG TADPOLE. G.R. Davis* and P.B. Farel (SPON: F.L. Eldridge). Dept. of Physiology, Univ. N. Car. Sch. of Med. Chapel Hill, NC 27514.

Spontaneous episodes of rhythmic bursting can be recorded from ventral rootlets of the isolated central nervous system of the bullfrog (Rana catesbeiana) tadpole (Stehouwer and Farel, 1980, 1981). This preparation has proved useful in studies of the development of coordination within and between hindlimbs (Stehouwer and Farel, 1983, in preparation). These studies have shown that hindlimb motoneurons exhibit coordinated fictive locomotor activity before the mesenchyme of the immature hindlimb bud has differentiated into contractile tissue. The early development of appropriately patterned activity in hindlimb motoneurons raises the question whether spinal circuitry that serves to coordinate activity between fore- and hindlimb motoneurons shows a similar early maturation. In the present work, we describe a preparation that will allow investigation of this question.

The entire CNS and pectoral region of tadpoles were removed and placed in a bath of oxygenated Ringer's solution. Suction electrodes were used to record from (a) medial ventral rootlets containing the axons of primary motoneurons (which innervate axial swimming musculature) and (b) lumbar lateral ventral rootlets (which innervate the hindlimb). In addition, the forelimb was dissected to allow recording from radial, ulnar, or brachial nerves.

Patterned bursting during episodes of fictive swimming could be recorded from forelimb nerves as early as st. XIII, despite the fact that the forelimbs do not emerge from the body until st. XX. Experiments in which the activity of fore- and hindlimb motor nerves is recorded simultaneously are in progress. A parallel study in which HRP was applied to the lumbar enlargement in order to retrogradely label brachial interneurons projecting to the lumbar enlargement suggests that the development of fore- and hindlimb coordination depends upon maturation of these propriospinal projections.

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267.10 STRETCH REFLEXES IN CHILDREN, B.M. Myklebust, G.L. Gottlieb, G.C. Agarwal, R. Penn, Dept. Physiology, Rush Med. Ctr., Chicago, IL 60612.

In the normal adult, activation of the soleus muscle (SOL) by tapping the Achilles tendon evokes EMG activity at monosynaptic latencies (30-50 ms), while the antagonist tibialis anterior (TA) muscle is electrically quiet. To quantify the magnitude of activation of antagonist muscles, we have computed the "TA:SOL reflex ratio" by measuring the ratio of the peak-to-peak amplitudes of the simultaneous tap-evoked EMG. The normal adult TA:SOL reflex ratio is usually less than 0.1 [Myklebust BM, Gottlieb GL, Penn RD, Agarwal GC: Ann Neurol 11:367-374, 1980].

To determine normal development of the stretch reflex, we computed the TA:SOL reflex ratio for normal neonates (1-10 days old) and in children (1 month to 14 years). In contrast to the normal adult the stretch reflex latency in neonates is 15-25 ms, and the TA:SOL reflex ratio is 0.14-3.3.

The TA:SOL reflex ratio at one month is similar to the neonate's. The trend of the population of children from 1 month to 5 years shows a decrease in TA:SOL from the neonatal range to the adult range. Serial testing of individual babies shows considerable variability in the ratio during the first year. By about the fifth year the reflex ratio is in the range of the normal adult.

We suggest that [1] the neonatal spinal cord is hyperexcitable with respect to the normal adult, and this gradually decreases during the first 5 years of development; or that [2] reciprocal excitation is a property of the neonatal spinal cord, and the mature pattern of reciprocal inhibition gradually develops during the first 5 years. The variability in the ratios may correlate with spinal organization as synaptic connections are complete. The normal changes in spinal cord circuitry which we propose may correlate with the acquisition of gross motor skills such as locomotion.

*This work was supported in part by the Foundation for Physical Therapy, National Research Service Award HL07320 and NIH Grant NS15630.

267.11 EFFECT OF BICUCULLINE ON MOTOR PERFORMANCE IN SPINAL CATS. G.A. Robinson, C.T. Leonard and M.E. Goldberger. Dept. of Anatomy, The Med. Coll. of Pennsylvania, Phila. PA 19129.

The quality of motor function of spinally transected (T12) cats, revealed by their ability to locomote on a motorized treadmill and perform placing and positive supporting reactions, depends on the age at which spinalization occurs. In general, the earlier the lesion is made, the greater the sparing and recovery of function. One possible explanation for these age-dependent differences is that the local circuitry responsible for generating locomotion might be more suppressed in cats transected as adults rather than as neonates. We therefore hypothesized that pharmacological blockage of inhibitory influences would result in facilitation of motor performance in cats transected as adults. We examined treadmill elicited locomotor performance, positive supporting responses and placing responses before and after the administration of bicuculline (a GABA blocking agent) in three groups of cats with chronic spinal transections made: 1) at one day post partum (1DPP); 2) at 11-17 DPP; and 3) in adulthood. Cats transected as adults responded to i.p. bicuculline (BCC) by increasing the number of continuous locomotor cycles elicited by the treadmill and by accomodating to treadmill acceleration and deceleration. Drug administration to the 1 DPP and 11-17 DPP groups failed to enhance their treadmill performance. The maximum force of extension during positive supporting responses was measured using a force plate. All groups showed increases in the force of extension after BCC administration. The thresholds for placing responses (recorded using a force transducer linked to a polygraph) were not consistently altered by BCC administration in any group. In other experiments naloxone HCl was administered i.p. to the same spinal animals. Preliminary evidence suggests that motor function is not altered by the drug. We conclude that the failure of adult-transected animals to show more recovery of locomotor function is due in part to the presence of local and specific inhibitory systems which develop to a greater extent than in neonatally transected animals.

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SYNAPTIC STRUCTURE AND FUNCTION II

268.1 NONSYNAPTIC SITES OF CATECHOLAMINE RELEASE. T. J. Sims. Department of Neurology, Stanford Univ. Sch. of Med. and VA Medical Center, Palo Alto, CA 94304.

Catecholamine (CA) neurons have been identified in the spinal cord of the axolotl salamander (Sims, T. J., J.C.N. 173:319-336, 1977). The cell bodies of these neurons are located in the ventral ependymal zone and have apical processes which contact the lumen of the central canal. Ultrastructural examination revealed dense core vesicles (DCVs) 100 to 140 nm in diameter within the cytoplasm of these neurons. Twenty-four hours following a single injection of .015 mg/100 g of estradiol 17-B cypinate (E-17B) the DCVs increased in number and there was a notable shift in the size of DCVs toward a smaller diameter vesicle (100 to 60 nm) with a smaller core. The random cytoplasmic distribution of DCVs seen in the normal female salamander changed to a predominance of DCVs located in the basilar cytoplasm of these neurons following E-17B treatment. DCVs in E-17B injected axolotls were often seen in clusters adjacent to the plasm membrane. Serial section analysis revealed that the clusters of DCVs were associated with small patches of dense undercoating on the plasma membrane. Coated vesicles were often seen fused with the plasma membrane in close proximity to the dense undercoating indicative of active membrane recycling at these sites. Glia cell membrane apposed the sites of dense undercoating with an extracellular cleft between the two membranes of 100 to 500 nm. No specializations were observed on the glia membrane opposite these sites. In salamanders with constant E-17B exposure for 72 hrs (injected once a day for 3 days) clusters of DCVs associated with presynaptic-like densities were no longer observed and the number of vesicles and their distribution appeared normal. This study shows that E-17B administration causes a transient change in the DCV population in a group of CA neurons. These neurons are probably involved in some aspect of neuroendocrine function. The transient appearance of presynaptic-like densities on plasma membrane, in association with DCVs, is interpreted as a morphological substrate for the nonsynaptic release of CA into the extracellular environment of the CNS. Supported by NSF ISP 801147, NIH NS-15320 and the Veterans Administration.

268.2 LIGHT AND ELECTRON MICROSCOPIC EXAMINATION OF SENSORY NEURONS AND MOTONEURONS MEDIATING THE TAIL WITHDRAWAL REFLEX IN APLYSIA. L.J. Cleary and J.H. Byrne, Dept. of Physiology, U. of Texas Medical School, Houston 77225

Sensory neurons mediating the tail withdrawal reflex are located in a homogeneous cluster in the pleural ganglion while the motoneurons are located in the pedal ganglion along the tract which arises from the pleural-pedal connective. We are interested in the structure of the connections between these neurons, especially as it relates to the mechanisms of synaptic plasticity. To begin, we are studying the geometry of the sensory and motor neurons and the ultrastructure of their connections.

Electrophysiological evidence indicates that both the sensory and motoneurons innervate the tail via the posterior pedal nerve, P9. We have confirmed this directly by injecting HRP into the cell bodies of both sensory and motoneurons since filled processes from both cell types are observed in P9. Motoneuron axons do not enter the pleural-pedal connective, however, indicating that the sensory neuron synpases are located in the pedal ganglion.

At the light microscopic level, a single process arises from the cell body of the pleural sensory neurons and projects to the pedal ganglion through the pleural-pedal connective. In sectioned tissue, the axons are observed to pass in a diffuse tract underlying the sensory cluster. These axons emerge from the pleural ganglion very close to the surface of the connective. Although no large branches were observed in the pleural ganglion, the proximal axons of each sensory neuron give rise to several fine processes, presumed to be dendrites. These dendrites may be the site of hyperpolarizing input evoked by stimulation of the skin or peripheral nerves.

Most sensory neurons continue through the pedal ganglion and out peripheral nerves without giving off a major branch. One cell was observed to branch in the pedal ganglion beneath the cluster of motoneurons. Many sensory neurons branch after exiting the pedal ganglion. This branching was associated with electrophysiological recordings of double spikes in response to electrical stimulation of P9.

At the electron microscopic level, numerous synaptic varicosities containing dense core vesicles are observed in close apposition to the sensory neuron processes in the pedal ganglion. These may be sites of modulatory input to the sensory neurons mediating heterosynaptic facilitation. We are currently examining additional aspects of the terminal ultrastructure of the sensory neurons.

268.3 ENHANCED CHLORIDE ACCUMULATION BY DENERVATED SKELETAL MUSCLE IN THE RAT. G.L. Harris, W.J. Betz, and J.H. Caldwell. Dept. of Physiol., Univ. of Colorado Sch. of Med., and Dept. of Molec. and Cell Biol., Natl. Jewish Hospital and Res. Ctr., Denver, CO 80262.

Rat lumbrical muscle fibers generate a steady electrical current which flows outward at the endplate and reenters in the flanking regions. The generation of the current can be accounted for by a nonuniform chloride conductance (G_{Cl}) along the length of the cell; G_{Cl} appears to be lower in the endplate region than elsewhere. The mechanism requires that the chloride equilibrium potential (E_{Cl}) be maintained at a value positive to the resting potential (V_m).

We examined the effect of denervation on the steady currents (measured with a vibrating probe). Since denervation is known to cause a large decrease in G_{Cl}, we predicted that the steady currents might decrease. However, the currents persisted, undiminished (1-35 days post-denervation). Moreover, the effects, on the steady current, of ion substitutes and drugs which block certain conductance or transport pathways suggested that the current generator mechanism did not change after denervation. One way to generate the same current across a decreased conductance would be to increase the driving force on Cl^- ($V_m - E_{Cl}$).

We used Cl^- selective microelectrodes to measure directly the intracellular Cl^- activity ($[Cl]_i$) in normal and denervated muscle. We found a marked increase in the ability of muscle fibers to accumulate Cl^- after denervation. In normal muscle, E_{Cl} was about 5 mV more positive than V_m, reflecting a $[Cl]_i$ of about 12 mM. Within 10 days following denervation, the difference between V_m and E_{Cl} increased almost 3-fold; $[Cl]_i$ increased to around 22 mM. Thus, the persistence of the steady current in denervated muscle can be explained by an increase in the driving force for Cl^- efflux. More generally, these results demonstrate that innervation exerts some control over intracellular Cl^- concentrations in mammalian skeletal muscle.

(Supported by NIH grants NS10207 (to WJB) and NS 16922 (to JHC), the Muscular Dystrophy Assoc. (to WJB) and an MDA postdoctoral fellowship (to GLH))

268.4 PHYSIOLOGY OF A SYMMETRICAL CHEMICAL SYNAPSE P. A. V. Anderson C. V. Whitney Lab. and Dept. of Physiology, University of Florida, St. Augustine, FL 32086

Neurons of the motor nerve net of the jellyfish Cyanea capillata are connected by morphologically symmetrical chemical synapses; both terminals contain apparently identical synaptic vesicles and these form two opposing clumps. This type of organization, which has also been demonstrated in other coelenterates and in molluscs, implies that the synapses are non-polarized and can conduct equally well in either direction. The synapses were examined electrophysiologically using whole cell, patch recording techniques. Two cells that could be seen to form a contact were "impaled" and one of the pair depolarized by intracellular current injection. Transmission between the cells occurs only when a spike is produced in the stimulated cell. There is no evidence of electrical coupling nor of unitary synaptic potentials in the absence of a pre-synaptic spike. The delay between the peak of the action potential and the onset of the post-synaptic response is close to 1 ms (mean = 976 µs). With cells that do not form a direct connection, the delay is 3 ms or longer. At the outset of any recording with a given cell pair, the typical response of the post-synaptic cell is an action potential. However, this gradually dissappears, presumably due to "washout", and a single, large (up to 60 mV) post-synaptic potential (PSP) remains. The amplitude of the PSP is dependent on the resting potential of the post-synaptic cell and over negative resting potentials the relationship is very linear, extrapolating to a reversal potential of close to zero mV (mean = +4.2 ± 7 s.d.). However, under normal conditions, reversal never occurred, primarily because of the powerful delayed rectifier present in these cells. This can be blocked by intracellular TEA, allowing better control of membrane potential at positive resting potentials. The synapses are truely symmetrical inasmuch as they are excitatory in both directions and the delays are almost identical. Reverberation across the synapse is prevented by the requirement of a spike for transmission. This preparation affords a unique opportunity for examining a somewhat novel type of synapse. Furthermore, because of the organization of the nerve net, intracellular recordings can be obtained from synaptic terminals, allowing accurate analysis of the mechanisms underlying synaptic transmission. Supported by NSF Grant BNS-8209849.

268.5 A RAPID METHOD FOR ISOLATION OF SYNAPTOSOMES ON PERCOLL GRADIENTS. J.A.P. Rostas, P.E. Jarvie*, J.W. Heath* and P.R. Dunkley*. The Neuroscience Group, Faculty of Medicine, University of Newcastle, N.S.W., Australia, 2308.

A rapid method has been developed for the preparation of synaptosomes from the post-mitochondrial pellet (P2) of rat cerebral cortex. The procedure replaces the usual sucrose or Ficoll gradients, which require long centrifugation times (2-5 hours) and large forces (50,000-200,000 x g) necessitating the use of an ultracentrifuge, by a four step Percoll gradient which requires only short centrifugation times (5 minutes) in a medium speed centrifuge (32,500 g). The P2 pellet is resuspended in 0.32M sucrose, containing 1 mM EDTA and 0.25 mM dithiothreitol, to a protein concentration of 4mg per ml. The suspension (2ml) is layered over a 4 x 2ml Percoll gradient comprising 2.5, 9, 15 and 22.5% Percoll made up (v/v) in resuspension buffer. The gradient is centrifuged at 4°C and 32,500 gav for 5 minutes in a Beckman JA-20 rotor (tube angle 34°). To assess the homogeneity and recovery of the subcellular fractions, quantitative electron microscopy has been undertaken and the distribution of protein, lactate dehydrogenase, cytochrome oxidase and specific phosphoproteins has been determined biochemically across the gradient. The synaptosome fractions obtained were shown to be viable in terms of noradrenaline uptake, as well as depolarisation-stimulated calcium uptake and protein phosphorylation. The procedure provides relatively homogeneous synaptosomes as well as myelin and mitochrondrial fractions, comparable with traditional gradient procedures. In addition, however, two major sub-populations of synaptosomes have been fractionated. The synaptosomes in the two fractions differ in their average diameter and mitochondrial content as well as in the amount of attached post-synaptic elements. Nerve terminals in vivo are different in size and contain different numbers of mitochondria per terminal, and this new procedure may provide a simple method for isolating two populations of functionally distinct synaptosomes.

268.6 STRUCTURAL AND ELEMENTAL CHARACTERIZATION OF ACETYLCHOLINE-RICH SYNAPTOSOMES FROM SQUID OPTIC LOBE. S.B. Andrews* and T.S. Reese (SPON: J. Fex). NINCDS, National Institutes of Health, Bethesda, MD 20205

Synaptosomes from the optic lobe of the squid are highly enriched in both acetylcholine and its specific enzymes and transport systems. However, electron microscopy (EM) has shown that these synaptosomes are quite heterogeneous. We have initiated a structural and compositional analysis of squid synaptosomes in order to determine which structural types retain significant functional properties. Freshly prepared squid synaptosomes were incubated for 30 min at 25°C in an artificial seawater (containing Ca++, choline, glucose and 10% BSA) prior to rapid freezing on a metal block cooled by liquid helium. Several classes of membrane-limited structures in freeze-substituted samples were candidates to be intact, functional synaptosomes; criteria for this selection included the presence and/or integrity of synaptic vesicles, mitochondria and cytoplasmic matrix, as well as the absence of damaged mitochondria or unrecognizable internal organelles. Measurements of internal potassium were used to determine which synaptosomes were functional on the assumption that high internal K implies the ability to reestablish a normal membrane potential. Thin ($\simeq$ 100 nm) cryosections were freeze-dried and examined unstained in an analytical EM in the laboratory of C.E. Fiori and R.D. Leapman at the NIH. Although these preparations did not have sufficient contrast for imaging of scattered electrons, they can be analyzed in this microscope because this instrument is equipped for digital image acquisition and quantitative analysis of characteristic x-rays and transmitted electrons. Concentrations of the major elements (Na, Mg, P, S, Cl, K, Ca) were determined directly from the digital image data in any structure which could qualify as a viable synaptosome on the basis of its K image. These structures were characterized by somewhat attenuated Na/K ratios (Na < 30 mmols/kg, K $\simeq$ 80 mmols/kg wet weight), by relatively low Ca (< 5 mmols/kg), and by easily detectable but variable amounts of P and S. However, 20% of these structures had a markedly higher (> 100 mmols/kg) concentration of K; this subclass also had S levels 25% greater than average. These preliminary results suggest that compositional, and, presumably, functional heterogeneity may correlate with structural heterogeneity in squid synaptosomes. It remains to make a positive morphological identification of the interesting subpopulation with high K levels approaching those expected in intact synapses.

268.7 INCORPORATION OF A VESICULAR ANTIGEN IN PLASMA MEMBRANE DURING EXOCYTOSIS OF NEUROTRANSMITTER. R. Robitaille*, J.P. Tremblay (SPON: R.F. de Estable-Puig). Lab. of Neurobiology, Laval Univ., Dept. of Anatomy, Québec, Canada, G1J 1Z4.

The incorporation of vesicular membrane in plasma membrane is studied at frog neuromuscular junction (nmj) during exhaustive exocytosis provoked by BWSV. This incorporation is studied with an antibody specific to vesicular membrane (J. Cell. Biol., 87, 98-103, 1980). The experiment is performed on cutaneus pectoris muscles dissected from small Rana pipiens and placed in chambers containing a saline solution. The 3 or 4 superficial layers of muscle fibers are cut to permit observation, with Nomarski optics, of nmjs on muscle fibers of the lower layer. The muscles are then incubated in a collagenase solution. Some muscles are then incubated in BWSV (0.2 gland/ml) to provoke the liberation of acetylcholine by exocytosis and therefore produce the incorporation of vesicular membrane in the terminal membrane (J. Cell. Biol. 52, 1-14, 1972). The other muscles are transfered to the standard saline solution. Immediately after these treatments, the muscles are fixed during four hours and rinsed overnight. The muscles are incubated overnight, at room temperature, with an anti-vesicular antibody (ANTI-VES). After rinsing, they are incubated during 2 hours with an HRP-conjugated swine anti-rabbit IgG (Cedarlane Lab., Hornby, Ont. Can.). The peroxidase is revealed according to the technique described by Towbin et al (PNAS 76, 4350-4354, 1979). For control, some muscles are incubated in normal rabbit serum instead of the ANTI-VES. Complete nmjs were observed with a light microscope at 400X. The labelling observed is restricted to muscles treated by both BWSV and ANTI-VES, indicating that there was an incorporation of vesicular membrane in the plasma membrane promoted by the BWSV and revealed by the ANTI-VES. The other types of muscles do not contain labelled nmjs, except a few muscles treated with ANTI-VES but not with BWSV. This labelling may result from spontaneous activity revealed by the ANTI-VES. This staining however, is lighter than that on the BWSV and ANTI-VES treated muscles. Our results suggest that the membrane antigen is present on the internal face of the vesicle before exocytosis and on the external face of the plasma membrane following exocytosis. They confirme the results of Von Wedel et al. (PNAS 78, 1014-1018, 1981).

268.8 HRP UPTAKE AND MORPHOMETRIC ANALYSIS OF VESICLES IN THE FROG SYMPATHETIC GANGLIONS FOLLOWING STIMULATION. C. Belhumeur* and J.P. Tremblay, (SPON: S. Radouco-Thomas) Lab. of Neurobiology, Dept. of Anatomy, Laval University, Québec, Canada, G1J 1Z4.

The afferent fibers of the right side IX and X sympathetic ganglions of five frogs were stimulated at 100 Hz during 20 minutes in vivo. Postganglionic compounds action potentials were recorded to verify the effectiveness of the stimulation. The ganglions were then fixed by perfusion with 2% formaldehyde and 1% glutaraldehyde and prepared for electron microscopy. Control ganglions from 5 other animals were also exposed for 20 min but not stimulated. About 18-20 synaptic profiles were photographed for each animal. The number of clear and dense core vesicles were counted separately for each synaptic profile. The profiles area was measured with an image analyser (Mop 3, Carl Zeiss). The numerical density on area (i.e. number of vesicles per um^2) was calculated separately for the clear vesicles and for the dense core vesicles for each synaptic profile. A two way analysis of variance and a Mann Whitney U test indicate no significant differences of the numerical density on area of clear and of dense core vesicles between the control and the stimulated animals. Dickinson-Nelson and Reese (J. Neuros. 3 (1983) 42-52) have recently reported a 50% reduction of the synaptic vesicles numerical density on area following stimulation at 10 Hz. Eight animals were therefore stimulated at 10 Hz for either 10 sec (as Dickinson and Reese) or for 20 min at 10°C or at 20°C. The ganglions of the other side of the same animal served as a control. Ten stimulated and ten unstimulated profiles were photographed for each animal. A two way analysis of variance and a Mann-Withney U test both indicate no significant difference between control and the stimulated numerical density on area of the clear and of the dense core vesicles. Although the stimulation at 10 Hz produces no significant morphometric changes, up to 30% of the clear synaptic vesicles in a profile were labelled with HRP following stimulation at 10 Hz in presence of extracellular HRP. This indicates that a rapid recycling of the vesicular membrane may be occurring in this system without any significant change in the numerical density of the clear vesicles. It is also possible that some of the vesicles are renewed by another process such as axoplasmic transport.

268.9 SYNAPTIC VESICLE RECYCLING STUDIED USING AN ENDOCYTOSIS MUTANT. J. H. Koenig and K. Ikeda, Div. of Neurosciences, Beckman Res. Inst. of the City of Hope, Duarte, CA 91010.

In the temperature sensitive mutant of Drosophila melanogaster, shibire^{ts1} (shi), endocytosis is normal at 18°C, but is reversibly blocked at 30°C. This blockage occurs at the stage where coated pits pinch off to form coated vesicles (Kosaka, T. & Ikeda, K., J. Neurobiol. 14:207-225, 1983). In shi neurons at 30°C, a blockage of synaptic vesicle recycling occurs, which leads to eventual vesicle depletion as exocytosis (transmitter release) proceeds normally. Furthermore, the accumulation of many pits along the plasma membrane, as well as some cisterna-like structures, is seen. This loss of vesicles from the synapse at 30°C coincides with a loss of the excitatory junction potential (ejp) and miniature excitatory junction potentials (mejp's) (Koenig et al., J. Cell Biol. 96:1517-1522, 1983).

Vesicle recycling was studied by first synchronizing the recycling process to the pit formation stage by exposure to 30°C, and then allowing the recylcing process to proceed by returning the temperature to 18°C. Immediately after the temperature is returned to 18°C, the accumulated pits are released to form the next structure in the recycling pathway, which results in a preponderance of such structures at this time. At a slightly later time, a preponderance of a subsequent structure in the recycling pathway is observed. Thus, by observing the recovering synapse at various times after the initiation of the recycling process, the various intermediate steps can be observed. The marker, horseradish peroxidase (HRP), was used to verify the progressive nature of the vesicle formation process. It was observed that the pits enlarge to form cisternae, many or all of which are continuous with the extracellular space. The cisternae then invaginate into themselves, forming crescent-shaped structures from the ends of which bud off vesicles. This often results in the formation of structures which resemble a necklace of beads (vesicles).

During these intermediate stages of vesicle formation, it was observed physiologically that "clustering" of mejp's occurs, i.e., the probabilty of several mejp's being released near simultaneously is increased. A possible correlation betwen the "clustering" phenomenon and the various intermediate structures in the recycling process is discussed. Supported by NIH grant NS18856.

268.10 **FREEZE-FRACTURE ANALYSIS OF ACTIVE ZONE ORGANIZATION AT IDENTIFIED FROG NEUROMUSCULAR JUNCTIONS.** Peter A. Pawson and Alan D. Grinnell. Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles, CA., 90024.

We are attempting to define mechanisms responsible for the 20-fold range in transmitter release per unit length that is observed in the sartorius muscle of the frog Rana pipiens. Last year we presented evidence indicating that an increased Ca^{2+} influx during the action potential may be largely responsible for the increased release (Soc. Neurosci. Abstr. 9, 1027). In an attempt to find an ultrastructural correlate for these findings, we have developed a method to study the active zone organization of individual, physiologically characterized nerve terminals.

Sartorius neuromuscular junctions(NMJs) were identified and their quantal contents determined. The muscle was then fixed and stained with nitroblue tetrazolium to reveal the terminal morphology. Identified junctions were drawn with a camera lucida and the endplate region of the muscle fibre was dissected out of the muscle. Identified endplates were then individually glycerinated, frozen in propane and stored in liquid nitrogen. Single fibres were fractured on a complementary replica device. In this way we have been able to correlate freeze-fracture replicas of identified nerve terminal active zones with the physiological data. We are presently refining the technique to optimize the amount of total terminal that we see in the freeze-fracture replica. While there is an extensive literature of freeze-fracture studies aimed at elucidating the release process at the frog NMJ, to date no one has performed a freeze-fracture analysis of individual, physiologically characterized NMJs. We expect that this work will help to elucidate the structural basis for the observed physiological differences in synaptic strength.

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268.11 IDENTIFICATION OF A TRANSMEMBRANE GLYCOPROTEIN SPECIFIC FOR SECRETORY VESICLES OF NEURAL AND ENDOCRINE CELLS. K.M. Buckley and R.B. Kelly. Dept of Biochemistry & Biophysics, University of California, San Francisco, Ca 94143

A monoclonal antibody (SV2) generated against highly purified synaptic vesicles from elasmobranch electric organ recognizes a ~100,000 Mr component of elasmobranch synaptic vesicles and crossreacts with synapses in virtually all parts of the mammalian nervous system. In addition, the antibody binds to several types of endocrine cells, including the adrenal medulla, intermediate and anterior pituitary, parafollicular thyroid cells, and pancreatic islet cells. The antigen is not detectable in liver or exocrine cells, including the salivary gland and the exocrine pancreas. In neuronal and exocrine cells in culture (PC12, AtT-20, GH3 and HIT cells), the antigen is localized to regions where secretory vesicles are known to accumulate as well as a perinuclear region which presumably represents the Golgi apparatus.

By immunoelectron microscopy and immunoprecipitation of intact vesicles we have determined that the antibody binds to the cytoplasmic face of synaptic vesicles. However, deglycosylation of the antigen, by enzymatic methods or protein synthesis in the presence of tunicamycin, results in a shift in the mobility of the material recognized in Western blots. These data suggest that this component is a transmembrane glycoprotein.

268.12 MULTIPLE FORMS OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II IN RAT BRAIN. T.L. McGuinness*, Y. Lai*, and P. Greengard (SPON: C.C. Ouimet). The Rockefeller University, New York, NY.

Calcium/calmodulin-dependent protein kinase II (CaM kinase II) is a multifunctional enzyme that may constitute up to 0.4% of total brain protein. It is composed of three autophosphorylatable subunits of M_r 50,000, 60,000, and 58,000, designated α, β, and β', respectively (McGuinness et al., FEBS Lett, 163, 329-334, 1983; Bennett et al., JBC, 258, 12735-12744, 1983). Studies by Walaas et al. (J. Neurosci., 3, 291-311, 1983) on the relative abundance of -M_r 50,000, 58,000, and 60,000 phosphoproteins in different brain regions suggested to us that different forms of CaM kinase II, which differ in their subunit ratios, might exist in various brain regions. To test this possibility, we purified CaM kinase II from two regions which showed the greatest difference in the relative amounts of the three phosphoproteins: the forebrain and cerebellum.

CaM kinase II was purified from the two sources by DEAE-cellulose chromatography, 35% ammonium sulfate precipitation, Sephacryl S-400 gel filtration, and CaM-affinity chromatography. The forebrain enzyme was purified 215-fold to >95% homogeneity and a specific activity of 4.4 μmol/min/mg. The cerebellar enzyme was purified 300-fold to ~80% homogeneity and a specific activity of 3.5 μmol/min/mg. The purified forebrain and cerebellar enzymes differed significantly in the relative ratios of the three subunits, as determined by ^{125}I-labeled CaM-binding, immunoblots, and densitometric scans of Coomassie blue and fast green stained SDS/polyacrylamide gels. The ratio of the α/β+β' subunits in the forebrain preparation was approximately 3/1 (β/β'~ 3/1), whereas in the cerebellar preparation the α/β+β' ratio was approximately 1/4 (β/β'~1/1). Immunoblots of forebrain and cerebellar crude homogenates showed similar subunit ratios. The corresponding M_r subunits of the two enzymes were shown to be identical by autophosphorylation, CaM-binding, immunoblots, and one- and two-dimensional peptide maps. The holoenzymes demonstrated similar behavior throughout the purification, similar hydrodynamic properties, and identical substrate specificities. The data indicate that different forms of CaM kinase II exist in different brain regions. Although the forebrain and cerebellar kinase each behaved as a single enzyme throughout the purification, it remains to be determined whether each preparation contains a single form or a combination of different forms.

268.13 PROFILES OF SPONTANEOUS RELEASE ALONG THE LENGTH OF FROG NERVE TERMINALS. A.J. D'Alonzo and A.D. Grinnell. Jerry Lewis Neuromuscular Research Center, UCLA, CA 90024.

We recently developed a new method to study evoked release of unit quanta along the length of frog neuromuscular junctions (D'Alonzo and Grinnell, Neurosci. Abstr. 8: 493, 1982). While this study did not reveal any gross non-uniformities in release, it did show that the quantal release/unit terminal length tended to be highest near or about the point of nerve entry and that it declined at the distal ends of the terminal.

We have now applied a similar analysis to spontaneously occuring miniature endplate potentials (mepps). Dissected frog cutaneous pectoris muscles were bathed in normal Ringer solution at 15°C. Two microelectrodes were inserted just beyond the distal ends of the terminal, and mepp amplitudes were simultaneously recorded from each electrode. The ratio of the mepp amplitudes seen at both sites was determined by an on-line micro-computer system. After a sufficient number of events were collected, a third, current passing, electrode was introduced and inserted at known locations along the muscle fiber, and the resulting amplitude ratios of the electrotonic pulses were similarly recorded and analyzed. In this manner, a calibration curve was generated which enabled us to correlate mepp ratios with the site of mepp origin. Endplates were visualized with nitroblue tetrazolium (NBT) and Karnovsky stains which allowed us to determine the release/length of a given segment of terminal. Preliminary results suggest that the profiles of spontaneous and evoked release are similar in that release/length is highest at the point of nerve entry. However, mepp release appears to be more uniform than evoked release.

A comparison of the release profiles of mepps and evoked quanta should help elucidate the underlying cause(s) of the observed non-uniformities. For instance, while the non-uniformity of evoked release may in part be attributable to the failure of action potential propagation along the length of the terminal (Mallart, Pflugers Arch. 400: 8, 1984), this mechanism cannot explain baseline non-uniformities in mepp release.

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268.14 ULTRASTRUCTURAL UNIFORMITY ALONG BRANCHES OF FROG MOTOR NERVE TERMINALS. M.J. Werle, A.A. Herrera, and A.D. Grinnell. Neurobiology Section, Dept. Biological Sciences, University of Southern California, Los Angeles, CA, 90089 (MJW & AAH) and Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles, CA, 90024 (ADG).

It is commonly assumed that synaptic ultrastructure and transmitter release properties are uniform along the length of motor nerve terminal branches. This is a critical assumption in: 1) physiological studies that use statistics to estimate release; 2) ultrastructural studies that infer an endplate's entire structure from samples taken at a few points; and 3) studies where transmitter release is normalized to nerve terminal length. In view of a recent report (D.F. Davey & M.R. Bennett, Dev. Brain Res., 5, 1, 1982) that there are structural gradients along nerve terminals in toad muscles, we felt it was necessary to examine this question more thoroughly using the frog neuromuscular junction (see A.A. Herrera & A.D. Grinnell, Soc. Neurosci. Abstr., 8, 492, 1982).

Nine endplates were studied in two sartorius muscles of adult Rana pipiens. Thin sections were taken every 6 μm at known locations, yielding between 40 and 105 cross-sectional views of terminal branches per endplate. Eighty terminal branches were examined, ranging in length from 20 to 246 μm. To compare terminals of different length, the distance between the end of the myelin sheath and the distalmost terminal tip was divided into bins of 10%. To look for gradients in real space, the same distance was divided into 30 μm long segments. Analyses of variance were performed on the raw data, on the average measurement for each bin, and on the sum of all data in each bin. We failed to find any spatial differences in active zone length, in 6 different measures of terminal size, in vesicle density, or in Schwann cell wrapping. There was a significant difference in the number of mitochondria per section, with more distally.

In a separate set of endplates we measured the spacing of cholinesterase stained synaptic folds from high resolution photomicrographs. There were no gradients in fold spacing and therefore in active zone spacing, assuming there is an active zone opposite each fold. In conclusion, we found no structural evidence for physiological non-uniformity along branches of motor nerve terminals in the frog. Supported by NIH grants NS18186 to AAH and NS 06232 to ADG.

268.15 VARIABILITY OF TIME CONSTANTS OF DECAY OF MIN. E.P.C.S. IN CONTROL SOLUTION AND AFTER CHOLINESTERASE BLOCKADE. M.I. Glavinovic (SPON: N. Lake). Depts. Anaesthesia Research & Physiology, McGill University, Montreal, Québec, H3G 1Y6.

As a result of spontaneous release of acetylcholine quanta from nerve terminals miniature end-plate currents (min. e.p.c.s.) which vary in amplitudes are observed. The origin of the variability of the quantal events is still largely unexplored, but recently it has been reported that under certain circumstances it can be altered. This study represents an attempt to cast a new light at the origin of the variability of the time constants of decay of miniature end-plate currents (τ's).

Experiments were done on the frog (Rana pipiens) cutaneous pectoris preparation at room temperature (19-21° C). The solution had the following composition (mM): NaCl 116, KCl 2.0, $CaCl_2$ 1.8 and was buffered by Tris Maleate buffer with pH adjusted to 7.1 - 7.3. Cholinesterase was blocked by applying neostigmine (3 µM).

In control solution, τ's are amplitude dependent (i.e. larger min. e.p.c.s. have longer τ's) but this dependence is not very pronounced. The amplitude dependence of τ's contributes to their variability as measured by the coefficient of variation (defined as the ratio of the standard deviation to the mean). Not surprisingly this contribution to the total variability of τ's is small. The variability of τ's occurs predominantly because of the scattering of τ's from the τ vs. min. e.p.c. curve.

The variability of τ's becomes more pronounced after cholinesterase blockade, mainly because of the marked increase in the amplitude dependence of τ's, which probably occurs because repetitive binding of ACh molecules is strongly dependent on the number of molecules released for quantum. The marked amplitude dependence of τ's indicated that if a quantum is the result of release of several vesicles, their release must be spatially very localized.

Increase in the standard deviation of the scatter of τ's from the τ vs. min. e.p.c. curve is comparable to the increase in the mean value of τ's, and therefore contributes only marginally to the increase in their variability. It probably occurs because the "critical area" affected by each quantum is enlarged and therefore with more variable density of ACh receptors.

Supported by MRC and MDA (Canada).

268.16 CAN A CHANGE IN THE SPATIAL DISTRIBUTION OF VESICULAR RELEASE ALTER THE AMPLITUDE OF QUANTAL EVENTS AND CONTRIBUTE TO THEIR VARIABILITY? D. Kneifel & M.I. Glavinovic (SPON: R. Chase), Depts. Anaesthesia Research and Physiology, McGill University, Montreal, Quebec, Canada.

The size of quantal events at the muscle end-plate is likely to be determined by various factors (a) presynaptic (vesicular volume, concentration of ACh in vesicles, fraction of vesicular ACh that is released) and (b) post-synaptic (density of ACh receptors on the post-synaptic membrane, size of the synaptic cleft). There is growing evidence that quantal events are spatially extremely localized. Therefore the spatial distribution of vesicular release can be another very important factor that influences the quantal size and therefore the variability of quantal events, if large gradients in the density of post-synaptic ACh receptors occur although over very short distances (i.e. in between active zones). Such gradients seem to exist but their magnitude is still controversial. There is evidence to suggest that in solution with elevated potassium (20 mM), after approx. 20 mins. the spatial distribution of vesicular release is altered and exocytosis also occurs in between active zones, at the sites of exocytosis that are presumably normally latent (Ceccarelli et al., J. Cell Biol., 1979, 81, 178-192). In the present experiments we examined how activation of these latent exocytotic sites affects the amplitudes of miniature end-plate currents (MEPC's).

The experiments were done on the frog (Rana pipiens) cutaneous pectoris preparation at room temperature (19-21°C) in normal Ringer. MEPC's were measured by a voltage-clamp technique.

As a result of elevated potassium (20 mM) the frequency of MEPC's rose to >100/sec within 5 min. The amplitudes of MEPC's slowly declined by an average of 15-25%, but the variability (estimated by the coefficient of variation) increased from about 25% to about 40%.

A small decrease in the mean amplitude and a more pronounced increase in the variability of MEPC's supports the idea that in elevated potassium exocytosis occurs not only at but also in between the active zones, opposite the post-synaptic membrane with reduced density of ACh receptors. Because of the nonlinear relationship between the MEPC's amplitude and the density of ACh receptors, the change in MEPC amplitude is likely to underestimate the changes in the density of ACh receptors.

Supported by NSERC (D.K.) and by MRC and MDA (M.I.G.).

268.17 DIFFERENTIAL DISTRIBUTION OF SYNAPSIN I ON SMALL SYNAPTIC VESICLES AND LARGE NEUROSECRETORY GRANULES. F. Navone*, P. Greengard, and P. De Camilli. CNR Center of Cytopharm., Dept. Med.Pharm., Univ. of Milano, Italy; Lab. of Mol. and Cell. Neurosci., The Rockefeller University, New York, USA.

Neuronal secretion involves release of molecules that range from small non-peptide molecules (classical neurotransmitters) to large proteins. In nerve endings, peptide neurotransmitters appear to be contained in dense-core vesicles of variable size, but larger than the typical 50 nm vesicles which are thought to represent quanta of classical neurotransmitters and which undergo repeated exo-endocytotic cycles in the terminal. Synapsin I is a major neuron-specific phosphoprotein which is present in all nerve endings, where it is specifically associated with the surface of synaptic vesicles [J.C.B. 96, 1337-1387 (1983)]. These findings suggest that Synapsin I may be involved in the regulation of synaptic vesicle traffic in the terminal. In our previous studies on the localization of Synapsin I, we confined our attention to the 50 nm vesicles which constitute the overwhelming majority of synaptic vesicles in the CNS. We have now investigated whether Synapsin I is also present on large dense-core vesicles. To do so we carried out Protein A-gold immunolabeling of synaptosomes from bovine hypothalami (a brain region rich in peptide neurotransmitters and dense-core vesicles) and of neurosecretosomes from the neurohypophysis (terminals of peptidergic neurons). 50 nm vesicles but not dense-core vesicles were highly labeled in the hypothalamic synaptosomes. Analogously, in pituitary neurosecretosomes, the peptide-containing neurosecretory granules were unlabeled, while label was present on the small vesicles in these endings. The function of these vesicles, which resemble typical 50 nm synaptic vesicles, is unknown. Our results indicate a) a biochemical similarity of small vesicles of the neurohypophysis to typical 50 nm vesicles of other nerve endings, and b) that in most and possibly all types of nerve endings, Synapsin I is specifically associated with the 50 nm secretory vesicles. While peptide-containing dense-core vesicles may be viewed as the neuronal equivalent of secretory granules of endocrine cells, 50 nm vesicles are secretory organelles peculiar to neurons. The association of Synapsin I, a neuron-specific phosphoprotein, with the 50 nm synaptic vesicle, a neuron-specific organelle, suggests that neurons possess a specialized secretory mechanism that is distinct from that associated with large secretory granules in neurons and other cell types.

268.18 PROTECTION BY PHYSOSTIGMINE FROM LETHALITY AND ALTERATIONS OF RAT SOLEUS NEUROMUSCULAR JUNCTION INDUCED BY SARIN. C.K. Meshul S.S. Deshpande* & E.X. Albuquerque. Dept. Pharmacol. & Exp. Ther., Univ. MD. Sch. Med., Baltimore MD 21201.

Pyridostigmine (PYR) and physostigmine (PHY) are potent reversible acetylcholinesterase (AChE) inhibitors and are known to interact with the acetylcholine (ACh) receptor-ion channel complex. Protection against a single lethal dose of the organophosphate sarin (130 µg/kg , subcut.) by pretreatment with a subcutaneous injection of PHY (100 µg/kg) or PYR (1600 µg/kg) was evaluated. Administration of a single, sublethal dose of sarin (90-100 µg/kg) induced typical signs of AChE poisoning. Electrophysiologic examination of the endplate region of the slow twitch soleus muscle 24 hrs after sarin injection showed spontaneous miniature endplate potentials of low amplitude, slow rise time and low frequency. Significant membrane depolarization of the surface fibers, low junctional ACh sensitivity and desensitization were also observed. Ultrastructural analysis of the endplate region showed disruption of post-junctional folds, distortion of sarcoplasmic reticulum and extensive subsynaptic vacuolization, leading to separation of the nerve terminal from the underlying muscle (Fed. Proc., 42,655, 1983). Pretreatment of rats with PHY, 30 min prior to the injection of an lethal dose of sarin protected 75% of the animals from lethality. This protection was increased to 100% after pretreatment with PHY and atropine (ATR, 500 µg/kg , subcut.). ATR alone did not afford any protection. Duration of symptoms after sarin challenge were reduced to 10 min after ATR plus PHY, 1 hr with PHY, compared to 4-5 hrs following a sublethal dose of sarin. Hematoxylin and eosin stained cross-sections of the soleus muscle biopsied at 24 hrs showed few necrotic muscle fiber lesions, compared to those seen in the soleus muscle of sarin (100 µg/kg) injected rats. Ultrastructural and electrophysiologic analysis of the soleus neuromuscular junction showed no significant alteration at 24 hrs, in contrast to that seen in sarin treated animals alone. Pretreatment with PYR showed only 10% survival of animals challenged with an lethal dose of sarin. In conclusion, only PHY appears to offer significant protection from lethality after sarin administration. Such effective protection could be related to the ability of the tertiary PHY to penetrate the central nervous system in contrast to little penetration by the quaternary compound PYR. (Supported by U.S. Army Med. Res. & Dev. Com. Contr. DAMD-17-C-81-1279)

268.19 QUANTAL SYNAPTIC CURRENT FLOW IN THE INTERFOLD OF THE VERTEBRATE NEUROMUSCULAR JUNCTION. J. Vautrin* and J. Mambrini* (SPON: ENA-SAS). Lab. Physiol. Géné., Univ. Paris XII, 94010 Créteil France.

The release of an acetylcholine (Ach) quantum by the nerve terminal induces a high concentrated Ach spot of about 1sq.µm at the surface of the postsynaptic membrane (Hartzell, H.C.,Kuffler, S.W. & Yoshikami, D., J. Physiol., 251:427,1975. and Wathey, J.C., Nass, W.M. & Lester H.A., Biophys. J. 27: 145, 1979). And it is well known that most postsynaptic apparatuses show infoldings of the subsynaptic plasma membrane set at *ca* 1µm intervals. So, the main part of each quantal current must flow in through one or two interfold slots before reaching the inside of the fiber body. The folds are sometimes deep and sit closer together, and could withstand the current flow in the subsynaptic apparatus. A similar problem was discussed for the extracellular current route in the synaptic cleft (Del Castillo, J. & Katz, B., Coll. Int. cnrs, Gif/Paris: 1955).

In order to appraise this resistance, models of the quantal current flowing through the interfold slots are proposed. The resistance of the interfold conductor is: Ri x K, where Ri is the resistivity of the interfold sarcoplasm and K the geometrical interfold coefficient. Two kinds of interfold morphology were considered: the cylinder-type interfold which is typical at the frog NMJ (Couteaux, R., J. Neurocytol., 10: 947, 1981), and the blade-type interfold where the interfold thickness is nearly constant. An expression of K is given for both interfold types. The compound interfolds were not studied.

Morphological parameters were picked up from literature electron micrographs showing interfolds pattern. Using the muscular sarcoplasmic resistivity measured already by numerous authors (100-200 Ω.Cm), the models indicate an interfold resistance between 500 KΩ and 2 MΩ. This resistance is in the range of the input resistance of the muscular fibers considered. The part played by this resistance and the potential thus generated between the interfold and the fiber inner part have to be explained.

268.20 SMALL GAP JUNCTIONS FOUND BY FREEZE FRACTURE IN HELISOMA GANGLIA. D.H. Hall, M.V.L. Bennett, and S.B. Kater (SPON:A. Chalazonitis). Dept. Neuroscience, Albert Einstein Coll. Med., Bronx, NY 10461, and Dept. Zoology, Univ. Iowa, Iowa City, IA 52242.

We have undertaken comparative studies of neuronal gap junctions in several invertebrates where electrotonic communication is well known physiologically, but where gap junctions have proven elusive in thin sections. Previously we reported the presence of small gap junctions and septate-like junctions between the neuronal membranes of the opisthobranch mollusc Navanax inermis (Hall, Spray and Bennett, J. Neurocytol., 12, 831, 1983). The small gap junctions were easily seen in freeze fracture replicas, but could not be demonstrated conclusively in thin sections.

Identified neurons in the buccal ganglion of the gastropod snail Helisoma trivolvis are known to form specific electrical connections in situ, and coupled pairs of neurons can pass the tracer Lucifer Yellow from cell to cell (Hadley and Kater, J. Neurosci., 3, 924, 1983). When the ganglia are axotomized and placed in organ culture, electrical connections are reestablished in hierarchical fashion (Bulloch and Kater, J. Neurophysiol., 48, 569, 1982).

In the present study small gap junctions were found in freeze fractured buccal ganglia and cerebral ganglia of Helisoma. These junctions have P-face particles and corresponding E-face pits, and are macular in shape. Particle packing is somewhat loose, and the average number of particles per junction is only 50 (N=24), somewhat less than observed previously in Navanax buccal ganglia. The failure to see gap junctions in thin sections of Helisoma buccal ganglia is not surprising because of their small size. It is not certain what proportion of the junctions seen in freeze fracture were neuronal, although in one case neuronal membranes were apparently involved, since the cytoplasm of one of the coupled elements contained numerous vesicles.

Septate-like junctions were not seen in either freeze fracture replicas or thin sections of intact ganglia. Other forms of membrane specializations are rather similar to those noted previously in Navanax, including tight junctions and orthogonal arrays of intramembrane particles in the outer glial sheath, spot desmesomes between glial cells surrounding the neuropil, and chemical synapses between neurons.

268.21 SYNAPTIC VESICLE MEDIATED CONTRIBUTION AND SYNAPTIC PROCESSING OF A NEURON-TYPE SPECIFIC SYNAPTIC CLEFT COMPONENT. P. Caroni*, S.S. Carlson , & R.B. Kelly. Dept of Biochemistry & Biophysics, Univ of California, San Francisco, Ca.

A library of monoclonal antibodies against highly purified cholinergic synaptic vesicles from the electric organ of D. Ommata has been generated. MAB1 binds to the luminal side of synaptic vesicles to a detergent-soluble glycoprotein that runs as a broad band of Mr larger than 200,000 on SDS-PAGE gels. Unlike 8 other synaptic vesicle specific monoclonal antibodies, MAB1 also recognizes a detergent-insoluble antigen in an extracellular matrix preparation (ECM) from the electric organ. The MAB1-antigen from the ECM sediments, under denaturing conditions, 7 times faster than its counterpart in the synaptic vesicle. Immunocytochemical methods demonstrate that the MAB1-antigen accessible extracellularly is found exclusively at synapses and is recovered in the ECM fraction. A MAB1-antigen form of intermediate molecular weight is transported anterogradely in the axons innervating the electric organ & is found in a synaptosomal preparation. MAB2 defines another synaptic vesicle antigen that is immunoprecipitated by MAB1. MAB2-antigen is transported exclusively from the nerve terminal to the neuronal cell bodies. MAB1 and MAB2 bind to antigens found exclusively in the neurons innervating the electric organ. A model for the synaptic vesicle mediated contribution and for the processing of a synaptic cleft-specific protein of unique distribution will be presented.

268.22 MODULATION OF PYRAMIDAL CELL FIRING BY ELECTRICAL FIELD EFFECT IN HIPPOCAMPAL SLICES. N. Ropert, Anaesthesia Research and Psychiatry Depts., McGill University, Montreal, P.Q., Canada.

Previous experiments have shown that the large field potentials recorded in the hippocampus generate transmembrane depolarizing potentials which can affect neuronal excitability (Taylor and Dudek, Science 218 (1982) 810; Taylor et al, Neuroscience 11 (1984) 101). The experiments described here show that synaptic potentials can modulate antidromic firing in a manner consistent with the idea that electrical field effects can modify cell excitability.

Experiments were done on thin tansverse slices of the rat dorsal hippocampus maintained in artificial CSF at 31°C (± 0.5). Extracellular field potentials were recorded from the CA1 stratum pyramidale. Bipolar stimulating electodes were placed in the alveus to activate pyramidal cells antidromically and in the stratum radiatum to evoke excitatory synaptic potentials (EPSPs) in the same neurones. Low frequency (< 0.5 Hz) test stimuli were applied to the alveus. They evoked antidromic negative population spikes (APS) with a peak latency of about 2 ms and a duration of about 2 ms. The maximal APS amplitude could reach 15 mV. The APS amplitude could be increased by as much as 300% by a conditioning stimulus (CS) applied to the stratum radiatum which was sufficient to evoke a small EPSP, but well below the threshold for an orthodromic population spike. The facilitation of antidromic cell firing depended also on the amplitude of the tested APS. When the APS was below a certain amplitude, it was not facilitated by a conditioning EPSP. Furthermore when the APS amplitude was maximal, it was also not facilitated. This strongly suggests that the enhancement of APS's of intermediate amplitude cannot be due to reversal of failure of somatic invasion by the antidromic action potential. Because of the very short latency and brief time course of the enhanced APS it is very unlikely that the facilitation could result from extracellular K^+ accumulation following single antidromic spikes.

In conclusion, this facilitation of stratum pyramidale neuronal firing is probably due to an electrical field interaction which seems to be a very significant mode of regulation of cell firing in the CA1 pyramidal cell layer of the hippocampus.

Supported by the Canadian MRC and the FRSQ.

268.23 EFFECTS OF MODERATE CHANGES OF $[K^+]$ and of $[Ca^{2+}]$ ON FIELD POTENTIALS IN HIPPOCAMPAL TISSUE SLICES IN VITRO. M. Balestrino*, P.G. Aitken, and G.G. Somjen (SPON: M.B. Abou-Donia). Dept. of Physiology, Duke Univ. Med. Center, Durham, NC 27710.

Effects of large changes of extracellular ion concentration on neuronal function have been studied in detail in the past. Our intention was to examine the quantitative relationship between neural function and changes of interstitial ion concentrations in the range likely to occur in intact brains under physiologic or pathologic conditions. Schaffer collaterals were stimulated, the responses in stratum radiatum and stratum pyramidale of CA1 zone were recorded concurrently, and input-output (I/O) curves were plotted. Raising $[K^+]$ in the bathing solution from 3.5 to 5.0 mM caused an increase of evoked population spike amplitude. Lowering $[K^+]$ from 3.5 mM to 2.0 mM caused a decrease of population spike amplitude. This confirms the effectiveness of these moderate changes of $[K^+]$ in altering hippocampal slice function. Raising $[Ca^{2+}]$ from 1.2 to 1.8 mM caused an increase of the EPSP evoked by a given amplitude of presynaptic volley (see also ref. 1); it also caused an elevation of post-synaptic firing threshold, gauged by the relationship of EPSP to population spike. As a result of these opposing effects, the overall synaptic I/O function, measured as the relationship of postsynaptic population spike versus presynaptic volley, was either moderately enhanced or not significantly changed. The effect of raised $[Ca^{2+}]$ on the relationship of presynaptic volley to stimulus pulse intensity was variable, causing an apparent increase, decrease, or no change in different slices. The same was true for the relationship of population spike versus stimulus intensity. Lowering $[Ca^{2+}]$ from 1.2 mM to 0.8 mM caused a decrease in population spike amplitude. This is in accordance with the decreased efficiency of synaptic transmission in this medium (1) which we were able to confirm in the present study. In addition, we found that in low $[Ca^{2+}]$ medium a given intensity of stimulation evoked a smaller postsynaptic population spike. The most likely explanation of the latter phenomenon is the depression of membrane action potential amplitude caused by low $[Ca^{2+}]$ that was reported by Frankenhaeuser (2).

(1) Dingledine & Somjen: Brain Res. 207:218-222, 1981.
(2) Frankenhaeuser: J. Physiol. 137:245-260, 1957.

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268.4 HIGH AFFINITY Mg^{2+}-DEPENDENT ATP-STIMULATED Ca^{2+} TRANSPORT ACTIVITY IN PURIFIED SYNAPTIC MEMBRANES. M.L. Michaelis and T.E. Kitos,* Ctr. for Biomedical Research, Univ. of Kansas, Lawrence, KS 66044.

Regulation of intra-neuronal Ca^{2+} concentrations is likely to involve a Ca^{2+} pumping ATPase which participates in restoration of low Ca^{2+} levels following depolarization. The presence of a specific Ca^{2+} pump in nerve terminal membranes has been difficult to establish due to the contamination of such fractions with membranes from other cellular organelles. We have recently described the properties of a $(Ca^{2+} + Mg^{2+})$-ATPase activity in synaptic plasma membranes. This enzyme differs from intra-neuronal $(Ca^{2+} + Mg^{2+})$-ATPases in its high sensitivity to vanadate (JBC 258, 6101-6108, 1983). In the studies described below, we have measured the high affinity ATP-stimulated transport of ^{45}Ca in synaptic membrane vesicles under conditions very similar to those used for assays of the enzymatic activity.

Highly purified synaptic membrane vesicles were loaded internally with 150 mM KCl-10 mM Tris/HCl, and the ^{45}Ca transport was measured in a medium containing 150 mM KCl-10 mM Tris/HCl-0.2 mM CDTA and various concentrations of ATP, free Mg^{2+}, and Ca^{2+}. Incubations were carried out at 37°C for 2 min and stopped by rapid filtration through Millipore filters (0.45 μ). The transport activity was linear for ≈3 min and was strictly dependent on the presence of Mg^{2+} ($K_{0.5}$ for Mg^{2+} ≈30 μM). The hydrolysis of ATP was required for transport as non-hydrolyzable ATP analogs did not stimulate Ca^{2+} uptake. Initial estimates of the affinities for the transport system for ATP and Ca^{2+} were: $K_{0.5}$ for ATP ≈10 μM and $K_{0.5}$ for Ca^{2+} ≈0.3 μM. Calcium transport in this preparation was quite sensitive to inhibition by very low concentrations of vanadate, with 50% inhibition observed at 2-3 μM vanadate. Since membrane vesicles capable of hydrolyzing ATP to transport ^{45}Ca to the internal compartment are likely to be vesicles with an inverted orientation, we have attempted to estimate what proportion of the total population are in this orientation. Initial estimates indicate 25-30% of the vesicles are inverted.

The ATP-dependent Ca^{2+} transporting activity in these vesicles exhibits many similarities to the $(Ca^{2+} + Mg^{2+})$-ATPase system. Thus it seems reasonable to assume that both the enzymatic and transport activities are being carried out by the same macromolecular entity present in the synaptic plasma membranes. (Supported by grants NS 16364, AG 01948, and by the Center for Biomed. Res. Univ. of KS.)

269 SYMPOSIUM: A CHOLINERGIC NEURON IN THE RETINA. K. Krnjević, McGill University (Chairman); R. Baughman, Harvard Medical School; M. Tauchi*, Massachusetts General Hospital; E. Famiglietti, Jr., Wayne State University; N. Daw, Washington University; R. Masland, Massachusetts General Hospital.

After many years of speculation about the role of acetylcholine (ACh) in the retina, recent information about the precise identity of likely cholinergic neurons provides new insight into the anatomical and functional organization of a retinal ACh-releasing system and its significance for the transmission of visual signals.

That a certain type of amacrine cell is probably a cholinergic neuron is strongly indicated by the results of biochemical, histochemical and immunohistochemical studies of the distribution of acetylcholinesterase and cholinacetyltransferase activity, as well as of high affinity choline uptake (Baughman). Selective mapping of these cells by intracellular dye injection has revealed an exceptionally wide and dense, overlapping dendritic arborization in the inner plexiform layer (Tauchi). On the basis of their morphology and situation, they have been identified as "starburst" amacrine cells that are symmetrically distributed on both sides of the inner plexiform layer, to the adjacent sublaminae of which they send their dendritic processes. Their principal input is from bipolar cells and other amacrines (probably including some that are GABA-ergic, but not cholinergic) and their output is exclusively to ganglion cells (Famiglietti). Because of the bistratified arrangment of ganglion cells of two functional (ON-OFF) types, the two subpopulations of "starburst" cholinergic neurons may selectively modulate ON or OFF ganglion cells.

These morphological findings are generally in keeping with the changes observed when cholinergic (apparently nicotinic) transmission in the rabbit retina is facilitated by physostigmine: that is an enhancement of either the spontaneous firing of "briskly" responding ganglion cells or the light-evoked responses of "sluggish" cells (Daw). They are also consistent with observations on the release of ACh from the isolated retina, though they do not explain why only transient light signals enhance ACh release, or the significance of the large spontaneous release that is neither Ca/Mg- nor K-sensitive (Masland).

270 SYMPOSIUM. THE MANY ROLES OF THE MUSCLE SPINDLE IN MOTOR CONTROL. D. G. Stuart, Univ. of Arizona (Chairperson); R. B. Stein*, Univ. of Alberta; A. Taylor, Sherrington School of Physiology; W. T. Thach, Jr., Washington Univ., G. E. Loeb, NIH-NINCDS.

Within the past several years, it has become possible to study the activity of muscle spindle afferents and, in some cases, fusimotor neurons during normal motor behavior. As new preparations have been developed, it has become increasingly clear that different motor control systems make different uses of the fusimotor apparatus to control the sensitivity of these proprioceptive sense organs. This is consistent with the recently appreciated diversity of independent fusimotor effects on afferent activity. At the same time, several different theories of motor control (such as length servos, follow-up servos, and stiffness regulators) have been put forward to account for general features of motor control.

This symposium will examine the motor control problems faced in such widely diverse tasks as walking (Stein), chewing (Taylor) and object manipulation (Thach). Speakers will consider how each theory of motor control might deal with the control problems inherent in the task they have studied and what predictions such theories make regarding the type of feedback information that should be coming from the spindles. These predictions will be compared and contrasted with the observed spindle inputs and outputs to determine the strengths and shortcomings of such general theories. A fourth speaker (Loeb) will discuss a newly formulated "task-group" hypothesis that links the behavior of spindles and other afferents to that of alpha and gamma motoneurons in the elaboration of motor acts that can be categorized in kinematic terms. A general discussion at the end of the session will focus on the value and universality of specific motor control theories and the possibility of identifying and testing more general notions of adaptability and optimization to account for the specialized control systems that appear to underly complex and well-coordinated behavior.

271.1 NEURITE OUTGROWTH IN VITRO FROM ONE BRANCH OF THE METACEREBRAL CELL OF APLYSIA IS REDUCED WHEN THE OTHER BRANCH HAS FORMED CHEMICAL CONNECTIONS. S. Schacher. Center for Neurobiology & Behavior, Columbia University, College of Physicians & Surgeons, and New York State Psychiatric Institute, New York, N. Y. 10032.

During development and regeneration extensive elimination or rearrangement of processes and synaptic connections has been observed. Although several mechanisms have been proposed to account for plasticity in the organization of the nervous system, the way in which individual neurons regulate the "survival" of some structures and the "elimination" of others is not clear. To explore this question, I have developed an in vitro system where neurite outgrowth and synapse formation at different branches of a single identified neuron can be examined under controlled conditions.

The metacerebral cell (MCC) soma of Aplysia was isolated from the cerebral ganglion of juvenile animals along with its bifurcate axon and maintained in culture (see Schacher and Proshansky, J. Neurosci., 3:2403, 1983 for details on culture): a) with no targets, b) with an identified buccal neuron (B1 or B2) placed near the large diameter cerebral- buccal connective (CBC) branch which innervates buccal ganglion neurons, or c) with the same buccal neuron cells placed near the stump of the small diameter posterior lip nerve (PLN) branch. Within 24 hrs processes emerged from the stumps of the MCC branches and in the presence of buccal neurons formed interconnecting networks.

After five days, the two branches showed differential capacities for the formation of detectable chemical synaptic connections. Over 90% of the cultures (19 of 21) with MCC-buccal neuron contact via the CBC branch showed chemical connections with properties identical to that seen in vivo for this connection. In contrast, chemical connections were present in only 20% of the cultures (5 of 25) with MCC-buccal neuron contacts via the PLN branch.

The formation of a chemical connection between the MCC and buccal neuron via the CBC branch was associated with a marked reduction in neurite outgrowth from the PLN branch compared to neurite outgrowth from the PLN in the absence of any target or when the buccal cell is placed by the PLN. Neurite outgrowth from the CBC branch was not significantly affected by the presence of a buccal neuron at either branch.

These results suggest that neurite outgrowth from one branch of a neuron can be down-regulated selectively as a consequence of appropriate target interaction at another branch. Since this simple in vitro system consists of only two neurons, it will now be possible to explore the signals and underlying mechanisms by which neurons regulate growth from different branches as they seek to form chemical connections with appropriate targets.

271.2 ELEMENTARY NEURAL CIRCUIT OF APLYSIA GILL-WITHDRAWAL REFLEX RECONSTITUTED IN CELL CULTURE SHOWS HOMOSYNAPTIC DEPRESSION AND 5HT-INDUCED FACILITATION. S. Rayport and S. Schacher. Center for Neurobiology & Behavior, Dept. Psychiatry, Columbia University, College of P & S, and N. Y. State Psychiatric Institute, New York City 10032.

The cellular mechanisms underlying habituation and sensitization of the gill-withdrawal reflex in Aplysia are contained within a relatively simple neural circuit in the abdominal ganglion (Kandel and Schwartz, Science, 218:433, 1982). LE sensory cells make monosynaptic connections with major gill and siphon motor neuron L7 mediating the reflex. Facilitator cells synapse on the presynaptic LE cell modulating the reflex. Repeated LE spikes lead to homosynaptic depression of transmitter release and in the intact animal to habituation. Facilitator cell spikes or 5HT application produces heterosynaptic facilitation, giving rise behaviorally to sensitization.

To establish the elementary gill-withdrawal circuit in cell culture, we isolated LE and L7 cells from enzymatically digested abdominal ganglia (Schacher and Proshansky, J. Neurosci., 3:2403, 1983). Cells were placed in close proximity in polylysine-coated petri dishes containing Aplysia hemolymph and L15 medium. By five days there was an extensive neuritic network, and 80% of cultures had LE-L7 connections with synaptic latencies of 5 to 10 msec and PSP amplitudes of 0.4 to 20 mV. Repeated stimulation of LE cells once every 20 sec led to decrement in PSP size with kinetics similar to those in vivo. Bath application of 5HT to a final concentration of 10^{-5} M produced depolarization of both LE and L7, LE cell spike broadening, and a 2-3 fold increase in PSP size as in vivo.

To simulate the in vivo cell circuit, we utilized the serotonergic metacerebral cell (MCC) as a facilitator cell. The MCC produces a 5HT-mediated increase in cAMP leading to modulation of muscle activity (Weiss et al., J. Neurophysiol., 42:791, 1979) in a manner similar to facilitation of the LE-L7 connection. When MCCs were added to the cultures (with or without L7), trains of MCC spikes reliably produced depolarization of LE cells (10 of 10 preparations), variably produced spike broadening (2 of 4), and variably produced facilitation of the LE-L7 connection (3 of 6).

For the first time an identified neural circuit mediating behavioral plasticity has been reconstituted in cell culture. This system will foster subcellular exploration of behaviorally relevant synaptic plasticities. Observations over time of the same cultures may define an ontogeny for homosynaptic depression and heterosynaptic facilitation in culture; if this parallels the pattern in vivo (Rayport and Camardo, 1984), a next step will be an exploration of the cellular controls underlying emerging plastic mechanisms.

271.3 SYNAPTOGENESIS BY SINGLE IDENTIFIED NEURONS IN VITRO: CONTRIBUTION OF RAPDILY TRANSPORTED AND NEWLY EXPRESSED PROTEINS. R.T. Ambron, S. Rayport, and S. Schacher. Anatomy and Cell Biology and Center for Neurobiology and Behavior, Columbia University, P&S, New York, NY 10032.

Although the formation of specific synapses is crucial to the development of an integrated nervous system, there is no clear understanding of synaptogenesis at the molecular level. Recently it was shown that identified neurons of Aplysia form transmitter-mediated synapses in vitro (Camardo et al. J. Neurosci. 3:2614, 1983). Using the giant cholinergic neuron R2, we are attempting to identify two classes of protein: 1) those involved in mediating cell surface events during synaptogenesis; and 2) those that appear only after synapse formation. When R2's cell body is removed from juvenile animals and placed in culture it rapidly extends neurites. Studies using ^{35}S-Methionine indicate that R2 synthesizes over 400 polypeptides: many of these are subsequently transported into the neurites. We presume that proteins involved in contacting target cells will be rapidly transported and inserted into membranes of the nascent synapse (i.e. growth-cone). Analyses by 2-D PAGE indicate that 25 of the neuritic proteins correspond to components that are rapidly transported to R2's synapses in vivo. Three other proteins transported in vivo are not present in the growing neurites. Hence, R2 regenerating neurites in the absence of a target already has many proteins that are en route to fully functioning synpases. At least 7 of the 25 neuritic proteins appear to be constituents of the growth cone. This was determined by comparing the labeled proteins of R2's neurite with those found at the growth cones of RUQ cells growing in vitro. These cells have large growth cones that can be isolated in pure form (Flaster et al. these abstracts).

R2 will form chemical synapses onto LUQ neurons or L11 in vitro. The synapse onto LUQ cells is a typical cholinergic IPSP containing both fast and slow components (N=15). In contrast, R2 elicits a predominately non-cholinergic decreased-conductance EPSP in L11 (N=15). These results imply that R2 releases a transmitter in addition to ACH. We have identified two proteins whose appearance correlates with synapse formation. Protein A (78kd) was present in all 7 experiments in which synapses were formed but was absent when R2 was grown alone. Protein B (74kd) was only present in 5 of the synapsing R2s. We are now attempting to determine the location of these proteins in the cell.

271.4 BIOCHEMICAL CHARACTERIZATION OF GROWTH CONES OF IDENTIFIED NEURONS CULTURED IN VITRO. M.S. Flaster, S. Schacher, and R.T. Ambron. Center for Neurobiology & Behavior, and Dept. of Anat. & Cell Biol., Columbia Univ., College of P & S, and N.Y.S. Psychiat. Instit., New York, N.Y. 10032.

Growth cones (GCs) play important roles in neurite extension, pathfinding, and synaptogenesis. Little progress has been made toward understanding the molecular mechanisms underlying GC function. Since differential pathfinding or synapse formation may depend upon molecular features unique to GCs of particular types of neurons, identification of such molecules would be facilitated by the analysis of the GCs from a single neuronal type. Here we describe an in vitro system using giant Aplysia neurons where we isolate the GCs of a group of identified neurosecretory cells.

The Right Upper Quadrant (RUQ) neurons of the abdominal ganglion were isolated and placed in cell culture (Schacher and Proshansky, J. Neurosci., 3:2403, 1983). Within 24 hrs the RUQs sprout neurites with GCs that are large (up to 30 um), highly refractile, and somewhat ellipsoid in shape.

EM analysis of the GCs of RUQ cells shows them to be similar to GCs of other cells grown in vitro. They contain: 1) a central region extremely rich in a variety of membranous structures, 2) a distal, cortical region largely devoid of membranous structures, and composed of a largely uniform, fine matrix, and 3) a plasmalemma often showing features consistent with ruffling. The GCs lack oriented microtubules.

The GCs can be severed from their neuritic shafts and freed from the surface by gentle prodding, where upon they round up and are easily collected. Using linear 1-D SDS-PAGE and following a 24 hr incubation in ^{35}S-methionine, the patterns of labeled polypeptides extracted from the GCs of RUQ cells were compared to the patterns of labeled polypeptides obtained from the neurites of R2 and several other identified neurons grown in vitro. There are at least 10 polypeptides in the RUQ growth cones with molecular weights between 45 and 220 kD. Of these, 7 are major polypeptides present in the neurites of other cells. Two broad bands of molecular weights 70 kD and 78 kD are highly enriched in RUQ growth cones. An additional band (90 kD) is prominent in RUQ neurites and GCs but present in very diminished quantity in the neurites of other cells. There are several major polypeptides common to the neurites of all cells analyzed (including RUQ) that appear to be absent or at least very much reduced in the RUQ growth cones. The differences in polypeptide distribution detected using a relatively low resolution method suggest that this in vitro preparation will be very useful for identifying cell-specific growth cone markers.

271.5 THE FORMATION OF NEUROMUSCULAR CONTACTS BETWEEN EMBRYONIC CELLS OF XENOPUS LAEVIS DIFFERENTIATING IN CULTURE IN THE ABSENCE OF CALCIUM. L.P. Henderson, M.A. Smith* and N.C. Spitzer. Biology Department UCSD, La Jolla, CA 92093

Although substantial evidence indicates that functional acetylcholine (ACh) receptors are not needed for formation of neuromuscular synapses, recent findings raise again the possibility that the release of ACh and/or other vesicular contents might be required (Hume et al., 1983; Young & Poo, 1983). We have examined the role of calcium(Ca)-dependent impulse-evoked release of ACh in the formation of functional neuromuscular contacts.

Dissociated cell cultures prepared from Xenopus laevis embryos (stage 15) were maintained either in medium containing 10 mM $CaCl_2$ (controls) or in Ca-free medium containing 10 mM $MgCl_2$ and 1 mM EGTA. Spinal cord neurons differentiating in Ca-free medium are more numerous and extend significantly longer neurites than cells differentiating in control medium (Bixby & Spitzer, submitted). Despite this, both the number of neuron-myocyte contacts and the number of terminations of neurites on myocytes were reduced in Ca-free medium (70% and 33% of controls, respectively).

Intracellular recordings from neuron-myocyte pairs during perfusion with a standard saline revealed that functional synaptic contacts were formed in Ca-free medium, although with a reduced frequency (~ 30% of controls). Postsynaptic potentials elicited by neuronal action potentials were smaller than those observed in control cultures. In addition, the frequency of spontaneous potentials was significantly lower in Ca-free medium. 0 mM $CaCl_2$/10 mM $MgCl_2$ saline reversibly blocked impulse-evoked release from neurons grown in Ca-free medium. As with control cultures, both evoked and spontaneous potentials were blocked by d-tubocurarine.

Labelling with ^{125}I- or rhodamine-conjugated α-bungarotoxin or iontophoretic application of ACh revealed no ACh receptor clusters on contacted or non-contacted myocytes grown in Ca-free medium. All myocytes tested in control and Ca-free cultures were sensitive to ACh. No differences were found in sensitivity to bath applied ACh between myocytes in the two culture conditions.

Our results suggest that evoked, vesicular release is not required for the initial formation of neuromuscular contacts, although it may be involved in further maturation of synapses. Roles for spontaneous quantal or non-quantal release have not been excluded.

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271.6 Distribution of Acetylcholine Receptors within the Receptor Cluster at the Newly Formed Neuromuscular Junction in Xenopus Cultures. Yoshi Kidokoro, Barry Brass* and David Schubert.* The Salk Institute, San Diego, CA 92138.

During neuromuscular junction formation acetylcholine (ACh) receptors migrate in the membrane and accumulate at the nerve contact region. This process of nerve-induced receptor accumulation is conveniently reproduced in Xenopus nerve-muscle cultures. The excellent visibility of cultured cells provides a unique opportunity to study the underlying mechanism. Recently we have tested a diffusion trap model which was proposed in 1976 by Edwards and Frisch to account for receptor clustering. In this model freely diffusing ACh receptors are trapped at the junctional region and eventually form high receptor density areas along the course of nerve contact. We observed that during cluster formation small clusters initially emerge from the background at the nerve contact. These clusters increase in size and number, and fuse to form larger clusters. Diffusely distributed ACh receptors move laterally in the membrane at a speed fast enough to account for the rate of cluster formation following innervation and the rate of cluster dispersal after denervation. These observations are compatible with the diffusion trap model. In these experiments the rate of receptor accumulation and dispersal was calculated assuming that the receptor density within the cluster is homogeneous and that there are 1,000 α-bungarotoxin binding sites/μm2.

In the present study we have tested the validity of these assumptions by scanning the optical density of photographic negatives obtained from innervated cells stained with tetramethyl rhodamine conjugated α-bungarotoxin. Since the grain density on the film is non-linearly related to the fluorescence intensity, we have produced a curve which correlates the grain density and the fluorescence intensity. Thus we are able to assess the relative fluorescence intensity at the newly formed neuromuscular junction with an excellent spatial resolution. We found that the fluorescence intensity is not homogeneous within and among the clusters. Within a cluster the receptor density was highest at the middle and declines toward the edge. The larger clusters have the higher densities of receptors at their middle than smaller ones, and the large clusters are often composed of multiple peaks separated by 2 to 4μm. This observation is compatible with our earlier finding that large clusters are formed by fusion of smaller speckles. We have also examined the distribution of newly inserted receptors and shown that their distribution is similar to that of old ones. We will discuss the contribution of preferential insertion of new receptors at the junctional sites.

Edwards, C. and H.L. Frisch (1976) J. Neurobiol. 4, 277-381.

271.7 RELATIONSHIP BETWEEN JUNCTIONAL ACETYLCHOLINE RECEPTOR (J-AchR) CLUSTERING AND Dolichos Biflorus AGGLUTININ (DBA) RECEPTORS IN REGENERATING SKELETAL MUSCLE. F.M. Hansen-Smith* (SPON: S.R. Barry). Depts. Physiology, Anatomy/Cell Biology, Univ. of Michigan, Ann Arbor, MI 48109.

Previous studies have shown that when skeletal muscle is injured the original J-AchRs are destroyed. However, during regeneration of the muscle fiber J-AchRs are reconstituted at the site of the original synapse, even when nerves are absent. It has been postulated that the synaptic basal lamina may mediate the expression of this phenomenon. The purpose of the present study was to determine whether the distribution of α-linked N-acetyl-D-galactosamine, a specific constituent of the post synaptic basal lamina, correlates with the distribution of the J-AchRs during regeneration. As a model for regeneration, the sternohyoid muscle from 200g rats was transplanted into a host site in the hindlimb. The sciatic nerve was severed to prevent innervation. Grafts were examined 1-60 days postoperatively. Rhodamine-conjugated α-bungarotoxin was used to detect AchRs in frozen sections. Biotinylated DBA conjugated to FITC was used to detect the α-linked N-acetyl-D-galactosamine (DBA receptors). Reconstructed J-AchRs were first detected in 4-day grafts. DBA co-localized with many of the J-AchRs, but was patchier and more dispersed than in controls. By 7 days DBA was undetectable at many of the JAchRs and patchy in the remainder. DBA was never associated with the extra-junctional AchRs that appeared during early regeneration. J-AchRs were present in 14-60 day grafts, but DBA was undetectable at these sites. However, when the sciatic nerve was left intact, the original or ectopic sites became innervated. DBA was associated with the J-AchRs in these grafts. This study suggests that 1) DBA receptors are not required for JAchRs to persist, but a role in J-AchR clustering cannot be excluded; 2) the initial appearance of junctional DBA receptors in regenerating muscle may be nerve-mediated.

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271.8 ACETYLCHOLINE RECEPTOR mRNA IS CONCENTRATED IN SYNAPTIC REGIONS OF ADULT MUSCLE FIBERS. J.R. Sanes and J.P. Merlie*. Depts. of Physiol. and Pharmacol., Washington Univ. Sch. of Med., St. Louis, MO 63110.

Acetylcholine receptors (AChRs) are highly concentrated in the postsynaptic membrane of the neuromuscular junction. As neuromuscular junctions develop in vitro, AChRs throughout the muscle membrane redistribute to form AChR-rich postsynaptic patches (Anderson and Cohen, J. Physiol. 268:757, 1977). In addition, new AChRs are preferentially synthesized or inserted near synaptic sites (Fischbach, Role, and O'Brien, this vol.). Similarly, maintenance of postsynaptic specializations on adult muscle fibers may involve directed redistribution and/or preferential local synthesis of AChRs. We provide evidence for the latter possibility by showing that mRNA encoding AChR is concentrated in synaptic regions of innervated adult muscles.

Mouse or rat diaphragms were dissected into synapse-containing and synapse-free regions (e.g., Hall, J. Neurobiol. 4:343, 1973), and a poly A+ RNA (mRNA-rich) fraction prepared from each. Equal amounts of RNA from the two regions were then fractionated by gel electrophoresis, transferred to nitrocellulose paper, and incubated with ^{32}P-labeled cDNA complementary to mRNA encoding AChR α-subunit (Merlie et al., PNAS 80:3845, 1983) or skeletal muscle actin (Caravatti et al., J. Mol. Biol. 160:59, 1982). Subsequent autoradiography allowed quantitation of mRNA levels. In 3 experiments the ratio of AChR mRNA abundance in synapse-containing and -free samples averaged 3.0. In contrast, actin mRNA was evenly distributed between synaptic and nonsynaptic regions (ratio=1). We conclude that AChR mRNA is present throughout the muscle fiber, but is concentrated near synapses. Cytological methods will be required to determine exactly where AChR mRNA is concentrated. However, since only ~1% of all muscle fiber nuclei (or ~3% in the synapse-containing sample) directly underlie postsynaptic membrane, AChR mRNA levels near synaptic nuclei might be ~100 fold higher than near nonsynaptic nuclei. Our results thus raise the possiblity that synaptic and nonsynaptic nuclei within a single muscle fiber's cytoplasm differ in their patterns of gene expression. (Supported by NIH and MDA).

271.9 THE CONTRIBUTION OF NEW AND OLD ACETYLCHOLINE RECEPTORS TO NEWLY FORMED POSTSYNAPTIC RECEPTOR AGGREGATES G.D. Fischbach, L.W. Role, R. O'Brien* and V. Matossian* (SPON: J. Dubinsky). Dept. of Anat. & Neurob. Washington Univ. Sch. of Med., St. Louis, MO 63110.

Clusters of acetylcholine receptors (AChRs) appear at newly formed chick nerve-muscle synapse in vitro within hours after nerve-muscle contact. AChRs in embryonic myotubes are synthesized and degraded rapidly and are mobile in the lipid bilayer. Therefore, nerve-associated receptor patches (NARPs) might form by accumulation of receptors synthesized after nerve muscle contact or by migration of 'old' AChRs exposed on the myotube surface prior to contact.

The mechanism of NARP formation was studied by: (1) labeling AChRs in a culture of chick myotubes with rhodamine conjugated α-bungarotoxin (RH-BTX) for 1 hr at 37°C, (2) adding dissociated chick ciliary ganglion neurons (along with unconjugated BTX) and then after 8, 11 or 17 hrs, labeling all AChRs with a monoclonal antibody directed against the α-subunit of the AChR, Mab 35 (gift of J. Lindstrom). The Mab was visualized with a Fl conjugated second antibody. The contribution of AChR present on the cell surface before neurons were added was taken as the ratio of RH-BTX to Fl-MAB 35 staining; the contribution of AChR inserted into the membrane after the addition of neurons was obtained by subtraction. Fluorescence intensity was quantitated with a SIT video camera connected to a Grinnell image analyzer and a PDP 11/44 computer. The digitized image of the NARP was enlarged so that the intensity of more than 200 points within the cluster was measured with 8 bits of resolution. This detection system was linear in the range of fluorescence intensities studied and control experiments reveal that we can detect differences in fluorescence intensity of less than 10%.

Within the first 24 hrs, the majority of receptors at NARPs are newly inserted (8 hrs: 62.2±4.0%, 11 hrs: 75.1-±4.5%, 17 hrs: 82.6±5.6%). In contrast, at non-nerve-associated clulsters (hotspots) only 16-26% of the receptors are newly synthesized and inserted over the same period of time. The rate of accumulation of new receptors at young NARPs is higher than the basal rate of receptor synthesis. In 3-day nerve-muscle co-cultures the percentage of new receptors at NARPs is similar to that predicted from basal rates of receptor synthesis (16-20%). Therefore, the induced synthesis is not maintained. In sum, both synthesis and migration of AChRs contribute to NARP formation but synthesis is more quantitatively important.

271.10 ACETYLCHOLINE RECEPTOR SYNTHESIS INDUCING FACTOR. T.B. Usdin* and G.D. Fischbach. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Acetylcholine receptors cluster at the site of nerve-muscle contact both in vivo and in vitro. Soluble factors released by motor nerves may play an important role in the regulation of ACh receptors as well as other postsynaptic proteins. We are pursuing the purification and characterization of a factor extracted from chick brain by measuring the change in the number of AChR's on the muscle surface with ^{125}I-α-bungarotoxin (α-BTX) (Jessell et al., PNAS 76:5397-5401, 1979; Buc-Caron et al., Dev. Biol. 95:378-386, 1983). With high specific activity α-BTX (~2000 cpm/fmole) we have been able to estimate the rate of receptor accumulation, after block with unlabeled α-BTX, on the surface of myotubes grown in 96-well micro-culture plates. We can reliably detect an increase in the rate of AChR incorporation of less than 50%. After extracting frozen brains in an acid cocktail the inducing activity was purified by ion-exchange chromatography (CM-Sephadex), and reverse-phase HPLC (Zorbax-C18 and Vydac-C4). On the analytical C4 column the inducing activity comigrated with a single symmetrical OD-210 peak. This material stimulates receptor synthesis by 400-500% after 24 hrs of incubation and based on dose-response curves represents approximately a 5000x purification. The activity is stable under our culture conditions since media containing the factor stimulates receptor synthesis when transferred onto new cultures even after a 24 hr pre-incubation with live muscle cell cultures. This C4 material can be further fractionated by ion-exchange HPLC and reverse phase HPLC on other supports. Efforts are in progress to assess the specific activity of this material, its effect on AChR aggregation, and to complete the purification.

271.11 ACETYLCHOLINE RECEPTOR SYNTHESIS: PURIFICATION OF INDUCING ACTIVITY FROM CHICK AND CALF BRAIN. D.G. Roufa*, M. Lerner* and G.D. Fischbach (SPON: J. Cohen). Dept. of Anatomy & Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

An early event in the formation of developing nerve-muscle synapses is the local induction of high density acetylcholine receptor (AChR) clusters at the site of nerve-muscle contact. The aim of the experiments reported here is to purify the active molecule(s) responsible for the increase in AChR number.

Saline extract of chick brain increases the rate of accumulation of the acetylcholine receptors (AChRs) in uninnervated skeletal myotubes in culture. Initial characterization of the activity from this extract showed that much of it was associated with acid soluble, trypsin sensitive, low molecular weight (less than 5 kd) material. Partial purification of this acid-soluble activity by HPLC was reported by Buc-Caron et al. (Dev. Biol., 1983), and further purification by HPLC is underway. We report here purification of the activity by an alternative protocol.

The inducing activity was monitored by a rate assay, that is, all pre-existing AChRs on the surface of cultured skeletal myotubes were blocked with αBTX; unbound αBTX was removed and the cultures grown for an additional 6 hrs; newly inserted AChRs are then labeled with ^{125}I-αBTX.

Supernatants of saline homogenates of frozen brains were fractionated with $(NH_4)_2SO_4$; most of the activity was recovered in the 45-55% precipitate. We followed the salt fractionation with preparative isoelectric focusing (IEF) in an LKB 110-ml column. The activity focused at an apparent pI of 5.0. Active fractions from IEF column were further chromatographed on octyl sepharose (OS); activity dissolved in 5% acetic acid adsorbed to the column and was eluted with 5% acetic acid plus 50% acetonitrile (ACN). Preparative gel electrophoresis of active fractions from IEF or OS column on 15% SDS-polyacrylamide gels (PAGE) and electroelution in a Savant preparative PAGE apparatus yielded active fractions following dialysis to remove SDS. The active fractions eluted approximately midway between the dye front and a 6 kd band whether a 15% or 15%+8M urea PAGE were used, indicating that the size of the active molecule(s) is less than 6 kd. About 6% of the initial activity was recovered and we are currently analyzing the composition of this fraction. In addition, we have obtained essentially the same results starting with saline extracts of calf brain and attempts to scale up the procedures are underway.

271.12 AChR-AGGREGATING EXTRACTS OF EXTRACELLULAR MATRIX-RICH FRACTIONS OF TORPEDO ELECTRIC ORGAN GENERATE ANTISERA THAT BIND SPECIFICALLY AT THE NEUROMUSCULAR JUNCTION. Justin R. Fallon*, Ralph M. Nitkin, Bruce G. Wallace, and U.J. McMahan. Dept of Neurobiology, Stanford University School of Medicine, Stanford, California 94305.

Molecules associated with the extracellular matrix at the neuromuscular junction in skeletal muscle direct several aspects of neuromuscular regeneration including the aggregation of acetylcholine receptors (AChRs) on myotubes. We have previously reported our progress toward identifying and purifying AChR-aggregating molecules from the electric organ of Torpedo californica (Nitkin et al, Cold Spring Symp XLVIII 1983). Our approach has been to make extracellular matrix-rich fractions from this tissue, solubilize the active components, and purify them on the basis of their ability to cluster AChRs in vitro.

Here we describe immunohistochemical staining of frog skeletal muscles using antisera raised against two of our fractions: an antiserum against the crude, soluble fraction and an antiserum against our most purified (>1000-fold) fraction, both of which block and immunoprecipitate the AChR-aggregating activity. We used frog muscles because the simple and orderly pattern of innervation makes it easy to locate junctions in histological sections and because AChR-aggregating molecules are known to be present in the extracellular matrix of these muscles.

When antiserum raised against the crude fraction was incubated with frozen cross-sections of muscles in conjunction with indirect immunofluorescence, staining of the extracellular matrix was seen in both junctional and extrajunctional regions of the muscle, but no staining of the muscle cell cytoplasm was observed. When antiserum against the more purified fraction was used, staining was specifically localized to the junctional region, co-localizing with α-bungarotoxin staining of AChRs. We conclude that as we purify for AChR-aggregating activity from the electric organ we purify for antigens that are situated specifically at the neuromuscular junction. We are presently generating more specific antisera to investigate whether the AChR-aggregating molecule from Torpedo electric organ is related to the molecules present in the frog synaptic extracellular matrix that direct AChR aggregation on regenerating myotubes.

This research was funded by grants from Muscular Dystrophy Association and NIH (NS 16440 and NS 14506) and postdoctoral fellowship from American Heart Assoc, Calif Affiliate to RMN.

272.1 PRENATAL EXPOSURE TO NICOTINE ALTERS SEXUAL BEHAVIOR OF MALE RATS AT ADULTHOOD. J. F. Rodriguez-Sierra and S. E. Hendricks, Dept. of Anatomy, University of Nebraska Medical Center, Omaha, NE 68105.

Pregnant rats were implanted with osmotic minipumps on day 12 of gestation. The minipumps delivered approximately either 25 or 50 mg/kg body weight/day at a rate of 1 l/hr for 7 days. This treatment did not alter significantly the body weight gain seen in pregnancy nor did it affect the length of gestation. All pups were cross-fostered at birth to normal mothers. Animals were tested throughout infancy for reflex and motor development and somatic growth. At 7 weeks of age, the animals were castrated, given a two-week recovery period and tested weekly for female sexual behavior. The animals were injected with estradiol benzoate (100 g/kg BW) two days prior to testing and progesterone (1 mg/rat) 5 h prior to testing. Testing was conducted during the dark-phase of the light-dark cycle of the animals using a sexually vigorous male rat to mount the tested animals. After the female sex behavior tests were completed, the animals were tested twice (once per week) for male sex behavior using a female in heat as a stimulus animal. All male rats were then implanted with a 40 mm silastic capsule containing testosterone and animals were tested at one and two weeks after testosterone implantation. The nicotine-exposed pups were born weighing less than the control rats and this difference in body weights persisted throughout the entire experiment. In addition, the nicotine-exposed pups showed impaired coordination and development of reflexes. The control animals showed greater locomotion at 20 days of age, but any differences in amount of locomotion between the groups disappeared by 40 days of age. The animals exposed to the highest dose of nicotine showed a higher incidence of feminine sexual behavior than control animals. The animals exposed to the lower dose of nicotine showed some feminine sexual behavior, but the differences were not statistically different. The nicotine-exposed animals showed a lower incidence of mounting, in the tests prior to testosterone treatment, than control rats. Nevertheless, testosterone exposure resulted in masculine behavior in all the groups. The results suggest that maternal consumption of nicotine can lead to alterations in the sexual differentiation of the fetus leading to femininization and/or demasculinization of the male.
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272.2 NEONATAL CASTRATION FAILS TO BLOCK DIFFERENTIATION OF THE DORSAL NUCLEUS IN THE MALE FERRET PREOPTIC/ANTERIOR HYPOTHALAMIC AREA. S.A. Tobet, D.J. Zahniser* and M.J. Baum. Dept. of Nutrition & Food Science, M.I.T., Cambridge, MA 02139 and Image Analysis Laboratory, Tufts-New England Medical Center, Boston, MA 02111.

A nucleus is present in the dorsal-medial part of the preoptic/anterior hypothalamic area (dnPOA/AH) of male ferrets which does not exist in females. Previous research showed that administration of testosterone, dihydrotestosterone, or estradiol to castrated adult male ferrets stimulated perikaryal size in the dnPOA/AH whereas these treatments failed to promote the formation of such a nucleus or stimulate perikaryal size in the comparable region of ovariectomized adult females. The present study was conducted to determine whether the presence of the dnPOA/AH in males depends on the action of testicular hormones during the early postnatal period, when testosterone acts in males to masculinize coital behavior. Male ferrets were castrated within 48 h of birth using ether and were implanted s.c. with Silastic capsules containing either testosterone or no hormone. Other males received sham operations neonatally and were later castrated at 10 weeks of age. When 12 weeks old all ferrets were anesthetized with nembutal and were then perfused via the heart with 0.9% saline followed by 10% neutral buffered formalin. Brains were stored in 10% formalin/25% sucrose solution prior to being sectioned in the coronal plane at 10 μm and 40 μm, alternately. Sections were stained with thionin. Analysis of 40 μm sections revealed no significant differences in maximal cross-sectional area of the dnPOA/AH among the three groups of males. Computer-assisted analysis of the 10 μm sections showed that the areas of cells in the dnPOA/AH were equivalent in the three groups of males, and in each instance were significantly larger than perikaryal of cells present in the dorsal-medial part of the POA/AH of females ovariectomized neonatally. The neonatal period during which testosterone masculinizes the coital behavior of males does not coincide with the time when hormones influence development of the dnPOA/AH. It seems likely that the formation of the dnPOA/AH in male ferrets depends on the prenatal action of testicular steroids.

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272.3 EFFECTS OF GLUCOCORTICOIDS ON PHENYLETHANOLAMINE N-METHYLTRANSFERASE(PNMT) IN CULTURES OF RAT SUPERIOR CERVICAL GANGLIA(SCG) AND MEDULLA OBLONGATA(MO) M.Bohn, C.Dreyfus, M.Goldstein & I.Black, Dept. Neurobiology & Behavior, SUNY, Stony Brook, NY 11794, Dept. Neurology, Cornell Med. Sch.,NY 10021 and Dept. Psychiatry, NYU Med. Sch., NY 10016.

PNMT is a specific phenotypic marker for epinergic-synthesizing (epinergic) cells. Previous studies in vivo have elucidated striking differences between peripheral and central epinergic cells in PNMT expression and regulation by glucocorticoids. Central PNMT is initially expressed 4 days earlier than peripheral PNMT. The development of central PNMT is not affected by decreasing glucocorticoid levels, whereas the development of peripheral PNMT requires glucocorticoids. However, we have observed that PNMT is increased in all epinergic cells following glucocorticoid treatment during specific development stages. To further investigate mechanisms involved in PNMT development, SCG and MO were studied invitro.

We reported previously that low levels of PNMT activity are present in cultures of newborn rat SCG. Dexamethasone (DEX 10^{-7}M) increases PNMT activity and staining in SIF cells via processes requiring protein and RNA synthesis. To determine whether this increase requires high affinity glucocorticoid receptors, we studied the effects of DEX 21-mesylate (DM) and cortisol 21-mesylate (CM), potent receptor antagonists in liver. Both DM and CM inhibited the DEX effect.

To examine central PNMT in vitro, embryonic day 13.5 myelencephalon was explanted for 3 weeks. PNMT was expressed in explants and PNMT-stained cells were observed. PNMT activity increased rapidly during the first 5 days in vitro and slowly during the next 2 weeks. Addition of DEX (10^{-6}M) during the 1st or 2nd week had no effect on PNMT. In addition, DM did not prevent the initial rise in PNMT activity during the first week. In contrast, DEX addition during the third week increased PNMT activity 35% above controls.

These experiments demonstrate that PNMT is expressed in peripheral and central neurons maintained in vitro. DEX increases PNMT activity in both MO and SCG explants. In SCG, this increase requires glucocorticoid receptor function. As observed in vivo, DEX increases PNMT during a limited stage in MO explants. In conclusion, explants are valuable for comparing factors regulating epinergic development in brain and periphery.

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272.4 THE INFLUENCE OF PERINATAL ANDROGEN EXPOSURE ON THE DISTRIBUTION OF DOPAMINERGIC CELLS AND FIBERS IN THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS (AVPv). R.B. Simerly, L.W. Swanson, R.J. Handa* and R.A. Gorski. Laboratory of Neuroendocrinology, Brain Research Institute and Department of Anatomy, UCLA School of Medicine, Los Angeles, CA 90024 and Salk Institute, La Jolla, CA 92112.

The AVPv, located at the rostral end of the third ventricle, appears to be involved in the control of luteinizing hormone (LH) secretion since lesions involving this nucleus disrupt phasic LH-release (Wiegand et al., Neuroendocrinol., 34:395). Further, compared to male rats, in the AVPv of females there appears to be a greater density of dopaminergic fibers and a larger number of dopaminergic cells (Simerly et al., Soc. Neurosci. Abstr., 9:1096). In this study, we used an indirect immunohistofluorescence method to evaluate the distribution of tyrosine hydroxylase (TH)-immunoreactive cells and fibers, and dopamine-B-hydroxylase (DBH)-immunoreactive fibers in the AVPv of female rats that were treated with testosterone propionate (TP) perinatally, postnatally, or left untreated. All of the postnatally TP-treated females failed to show a phasic pattern of LH-secretion in response to estrogen and progesterone, and both the perinatally and postnatally TP-treated females had polyfollicular ovaries at the time of gonadectomy. Perinatal TP-exposure reduced both the number of TH-stained cells and the density of TH-stained fibers to levels that were indistinguishable from those of males. In the postnatally TP-treated females the number of TH-stained cells was also completely masculinized, however, the TH-stained fiber density was not significantly affected. Although gonadectomy in the adult male resulted in a moderate increase in TH-stained fiber density, it did not significantly affect the fiber density in females, nor the number of TH-stained cells in either sex. The distribution of DBH-stained fibers in the AVPv did not appear to be altered in any of the treatment groups. These results suggest that the distribution of dopaminergic fibers in the AVPv may be sensitive to testosterone levels in the adult male, and although the critical period for the development of this fiber distribution may begin in the prenatal period, the number of dopaminergic cells in the AVPv can be completely sex-reversed by a single postnatal dose of TP and appears to correlate with a permanent disruption of the normal pattern of gonadotropin secretion. We wish to thank Dr. T. Joh and Dr. K. Helle for generous supplies of antisera. Supported by NIH grants HD01182, HD7728, and NS16686.

272.5 SEXUAL ACTIVITY AND SEXUALLY DIMORPHIC NUCLEUS VOLUME IN MALE RATS ARE CORRELATED WITH PRIOR INTRAUTERINE POSITION

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Both the ontogeny of male sexual behavior and the larger volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in males relative to that in females are presumably mediated by estrogen acting on the brain. Intrauterine position may influence these developmental processes, since rat and mouse fetuses appear to transmit steroids to contiguous fetuses. For example, the concentration of estradiol-17β in amniotic fluid is 50% higher in male mouse fetuses that develop between 2 female fetuses (0M males) than in males that develop between 2 male fetuses (2M males; F. vom Saal, et al, Science 220:1306, 1983). In this study the sexual behavior and SDN-POA volume of both male and female Sprague Dawley rats from known intrauterine positions were evaluated.

Adult 0M (N = 11) and 2M (N = 13) male rats were paired with an estrogen and progesterone primed, sexually-receptive female, and sexual behavior was recorded under red light until sexual satiety was reached (30 min without a mount). The 0M males ejaculated significantly more times (mean±SEM: 4.7±.4) than did the 2M males (3.2±.4; t-test; $p < .02$). 0M and 2M female siblings of these males (N = 13/Group) were ovariectomized in adulthood and administered s.c. injections of estradiol benzoate (10 μg) followed 48 hr later by progesterone (200 μg). Four hr after the progesterone injection the females were photographed in front of a grid while their flanks were palpated to induce lordosis. The intensity of lordosis was determined by measuring the height of a female's head and rump relative to the height of the back. The lordosis-intensity index was significantly greater for 0M females (5.0±.3) than for 2M females (3.8±.3; t-test, $p < .02$). The volume of the SDN-POA was analyzed in these rats as described by Gorski, et al. (J. Comp. Neurol. 193:529, 1980). The SDN-POA was significantly larger in 0M males ($4.41 \pm .29 \times 10^{-2} mm^3$) than in 2M males ($3.50 \pm .17 \times 10^{-2} mm^3$; t-test, $p < .01$), but the volume of the SDN-POA did not differ significantly in a sample of the females (0M females = $1.66 \pm .24 \times 10^{-2} mm^3$, N = 7; 2M females = $1.59 \pm .21 \times 10^{-2} mm^3$, N = 6). We propose that differences between 0M and 2M male rats in SDN-POA volume and sexual behavior are mediated by differential exposure to estrogen based on intrauterine proximity to female fetuses. Supported by NSF Grant BNS03714 and NIH grants RR07053, MH35079 and HD01182.

272.6 ANDROGEN FORMS SEXUALLY DIMORPHIC SPINAL NUCLEUS BY SAVING MOTONEURONS FROM PROGRAMMED DEATH.

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Perinatal androgen determines whether rats as adults will have the spinal nucleus of the bulbocavernosus (SNB) and the bulbocavernosus/levator ani (BC/LA) muscles which it innervates. Females are not normally exposed to androgen and so as adults lack BC/LA and have far fewer motoneurons in the SNB area than do males. Androgen could cause the sex difference in SNB number in 3 ways: 1) by augmenting neurogenesis in males. This alternative has been eliminated since SNB mitosis is complete before androgen secretion begins. 2) Androgen might direct the differentiation of immature cells into motoneurons, which then innervate BC/LA. The present results indicate that this is not the case, but rather that androgen recruits extant motoneurons to make up the SNB. These results also support the final alternative that 3) androgens form the SNB by rescuing motoneurons from programmed death.

During the 12th day of gestation (d12g), pregnant Sprague-Dawley rats were injected with tritiated thymidine (*TH), a specific precursor of DNA and hence an index of mitotic history. The radioactive labelling of spinal cell nuclei was autoradiographically monitored at various ages thereafter. By the day of birth (d1) many cells had clearly differentiated into large, multipolar, densely-Nissl-staining motoneurons, virtually all (95%) of which were densely labeled with the *TH. However, only 22% of the non-motoneuronal cells were heavily labeled on d1. Females injected with 1 mg testosterone propionate (TP) on d1, 3 and 5 had SNB cells in adulthood, 98% of which were heavily labeled with the *TH, indicating a mitotic history in common with d1 motoneurons but not with d1 non-motoneurons. Thus the androgen-induced SNB cells in these females were almost certainly derived from cells which had already differentiated into motoneurons.

Counts of L5 & L6 motoneurons in male, female and TP-treated female rats during development confirmed the remaining hypothesis that androgen spares SNB motoneurons from programmed death. There is already a sex difference in the number of motoneurons in the SNB region by d1pn. In confirmation of the hypothesis, the number of SNB cells continues to decline in females unless they are given TP. Since there is no difference in the number of non-SNB motoneurons in control females and those given TP, the androgen-induced check in the decline of SNB cells must be due to their rescue from programmed death.

# SNB cells ±SEM:	d1 of life	d6	d12
Males -	345.6 ±19.6	313.7 ± 2.8	325.0 ±17.0
Females -	170.0 ±22.9	118.3 ± 8.3	83.8 ± 8.0
Females +TP -	--	165.2 ±12.0	173.2 ±12.2

Supported by NIH grant # NS19790.

272.7 CASTRATION AFFECTS SINGLE ENDPLATES IN AN ANDROGEN-SENSITIVE MUSCLE OF THE RAT.

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The "levator ani" or dorsal bulbocavernosus (DBC) of the rat is a muscle which is absent in females and is highly sensitive to circulating testosterone in adult males. Both muscles and motoneurons for this muscle have receptors for androgens, and the muscle responds to castration with a decrease in weight, protein, soluble acetylcholinesterase (AChE, Tucek et al., J. Neurol. Sci. 27:353, 1976) and acetylcholine receptor number (AChR, Bleisch et al., J. Neurobiol. 13:153, 1982). These decreases can be prevented by treating castrates with testosterone. The number of muscle fibers in this muscle is not affected by androgens in the adult.

We now report that castration causes morphological changes at endplates in this muscle. Rats were castrated or sham-operated by the abdominal route. Ten weeks later, we prepared dissociated muscle fibers from the DBC muscle and stained them to demonstrate cholinesterase or labelled AChRs with I-125-α-bungarotoxin and prepared autoradiograms. Although muscle fiber diameters decrease substantially after castration, the dimensions of the endplate as revealed by AChE did not change in any obvious way. However, small ectopic spots of ChE were observed near 14 of 22 endplates from intact males, but near none of 20 endplates from castrates, suggesting that castration may decrease the rate of formation of terminal sprouts. The density of AChRs at endplates also decreased after castration by at least 33% ($p < 0.01$). Thus, castration causes changes at single endplates in this specialized, androgen-sensitive muscle.

We also report that 5 α-dihydrotestosterone (DHT), the non-aromatizable active metabolite of testosterone, can prevent the decrease in weight, protein and AChRs which occur rapidly after castration. Rats were castrated or sham-operated as above, and castrates recieved silastic capsules filled with DHT or cholesterol (as a control) 7 d later. Weight, protein, AChRs and AChE were measured 19 d after castration. Castration caused a drop in muscle weight, protein and AChRs (59%, 63% and 64% decreases). DHT reduced these decreases, so that muscle weight was 50% greater than in untreated castrates, while protein was 44% greater and AChRs were 68% greater. Neither castration nor DHT had any significant effect on total soluble AChE. Thus androgens, and not estrogen derived from testosterone, are apparently responsible for the testosterone-sensitivity of this muscle. (Funded by Rockefeller Univiversity)

272.8 A STEROID SENSITIVE ELECTROMOTOR PATHWAY IN MORMYRID FISH: ELECTRIC ORGAN MORPHOLOGY, ANDROGEN RECEPTOR BIOCHEMISTRY & STEROID AUTORADIOGRAPHY.

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Gonadal steroid hormones can induce a male-like Electric Organ Discharge waveform (EOD) among females and juveniles of mormyrid electric fish with a sexually "dimorphic" EOD. The male EOD is of longer duration and so lower power spectrum. We now describe possible anatomical and biochemical substrates for steroid effects on peripheral (electric organ) and central elements of the electromotor pathway.

The myogenic electric organ has disk-shaped cells or electrocytes with spike-generating anterior and posterior faces that determine the appearance of the EOD. For Brienomyrus brachyistius(Nigeria), the mean peak frequency of the EOD power spectrum is 4.2 and 1.3 KHz respectively for control and androgen-treated females. Spectral differences correlate with those in average electrocyte thickness (TT) and anterior face "thickness" (AF) (cf. Bass et al. Anat. Rec. V.205, 1983): TT and AF are respectively over 60% and 100% greater for androgen-females than controls. Cholesterol-females have a slightly lowered peak frequency (3.7 KHz) and increased TT (15%) and AF (24%). Anatomical differences may relate to changes in the electrocytes' spike-generating properties and so the EOD waveform.

The electric organs of 6 adult males were pooled to assay androgen binding activity (cf. Segil et al. Soc. Neurosci.Abstr.1983): cytosolic receptor concentration was 24.5 femtomoles of steroid specifically bound/mg protein(Kd≅1nM) compared to 4.5 fm/mg for "control" trunk muscle. Preliminary data indicate a sex difference in receptor binding.

Central androgen-binding sites were determined for 3H-dihydrotestosterone using autoradiography. For B. brachyistius (Gabon), labelled cells lie just lateral and caudal to "relay" cells of the medullary command nucleus which project to spinal motoneurons that innervate electrocytes. Labelled cells may project to neighboring relay neurons thereby affecting their excitation rate and, in turn, the electric organ's firing rate (i.e. rhythm).

In summary, the above data for the electric organ suggest it has evolved a sensitivity to steroid hormones that underlies the development of a sexually dimorphic EOD waveform. The discovery of androgen-binding cells in the central "command" zone may relate to the development of sex differences in the EOD rhythm as well.

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272.9 GONADAL-INDEPENDENT MODULATION OF LH SECRETION IN MATURING FEMALE RATS. H.F.Urbanski* and S.R.Ojeda*(SPON:E.D.Ross). Dept.of Physiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

The secretion of LH in prepubertal female rats is episodic and during the juvenile-peripubertal transition the afternoon pulse pattern changes. The amplitude of the LH pulses increases markedly and in some instances a 2-hour-long minisurge of LH secretion has been observed. Pulse frequency, however, remains constant. In the present study we sought to determine the extent to which these prepubertal alterations in LH secretion result from a centrally-originated, gonadal-independent, development. A total of 24 female rats were ovariectomized when 6-days-old and their plasma LH profiles were subsequently examined, either on days 28, 38 or 48; these time points corresponded to the juvenile, pubertal and adult phases of sexual maturation seen in intact female rats. Blood samples were obtained using an automated system which permits continuous sampling for more than 5 hours while simultaneously replacing the blood volume with a suspension of red blood-cells in artificial plasma. Mean plasma LH levels increased promptly after ovariectomy and remained at around 900 ng LH-RP-1/ml throughout development. Also no differences in the LH secretory pulse pattern were detected between any of the three age groups studied. Mean pulse amplitude was approximately 700 ng/ml regardless of the animal's age and the LH pulses occurred on average once every 30 minutes (a rate similar to that previously observed in intact prepubertal animals).

The results indicate that the juvenile female rat, in contrast to the primate, does not show a gonadal-independent decline in LH secretion. They also demonstrate that the prepubertal enhancement of afternoon pulse amplitude cannot be detected if the ovaries are removed during the neonatal period. It is suggested that the developing ovaries play a significant role in generating the peripubertal afternoon changes in LH secretory pattern. The central component in this mechanism may perhaps be uncovered by a short-term castration paradigm. (Supported by NIH Grant HD-09988-07, Project IV).

272.10 LOCALIZATION OF SOMATOSTATIN AND CHOLINE ACETYLTRANSFERASE IN THE INTERPEDUNCULAR NUCLEUS OF THE DEVELOPING CAT. B.J. Morley, K.M. Spangler*, E. Javel. The Boys Town National Institute for Communication Disorders in Children, Omaha, NE 68131.

Neurosecretory cells have been identified in the Interpeduncular nucleus (IPN) of the frog [1] and human [2]. These cells may contain somatostatin (SS) [3]. We now report that SS-immunoreactive perikarya and fibers have been localized in the IPN of the developing and adult cat. For these studies 20 cats varying in age from birth to 8 yrs were prepared for PAP immunocytochemistry. Plots were made of the cells and the intensity of SS-immunoreactivity was quantified by densitometer readings. In order compare the distribution of SS with that of choline acetyltransferase (CAT), two kittens were prepared for PAP immunocytochemistry and double-labeled with anti-CAT and anti-SS antisera (Immunonuclear, Inc).

The most significant observation that we made was a rapid increase in the concentration of immunoreactive SS in the developing kitten. The immunoreactivity appears to be maximum at about four months of age and to rapidly decrease thereafter. The increase was primarily the result of increased fiber staining while the decrease in adults was associated with a decrease in the number of immunoreactive cells and a decrease in the intensity of staining within individual perikarya.

If the cells which we described are homologous to the SS-containing perikarya observed in Rana, then there may be IPN cells in certain mammals that secrete SS. The IPN is located in close proximity to the interpeduncular cistern and opposite the median eminence, suggesting that the IPN might function as apart of an SS feedback system. If the intensity of immunoreactivity is an indication of functional activity, then this sytem may be most active during development.

[1] Kemali, M., Cell Tiss. Res., 178 (1977) 83-96.

[2] Kemali, M. and Casale, E., Z. Mikrosk.-anat. Forsch., 96 (1982) 591-599.

[3] Vandesande, F. and Dierickx, K., Cell Tiss. Res., 205 (1980) 43-53.

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OPIATES, ENDORPHINS, AND ENKEPHALINS: PHYSIOLOGICAL EFFECTS I

273.1 INTERACTION OF THE HYPOTHALAMIC NORADRENERGIC AND OPIATE SYSTEMS ON LH AND PROLACTIN SECRETION. C.A.Barraclough* and A.Akabori*(Spon: D.Ruchkin). Dept. of Physiol., Univ. of MD., School of Medicine, Baltimore, MD 21201.

Barraclough and Sawyer (1955) observed that morphine (M) blocks ovulation in proestrous rats and subsequent studies by others implicated the noradrenergic system in M suppression of phasic LH release. We have examined the effects of M sulfate (50 mg/kg s.c.) on norepinephrine (NE) and dopamine (DA) turnover in medial preoptic nucleus (MPN) and median eminence (ME) of estradiol (E_2)-treated ovariectomized (OVX) rats. M blocked LH surges and the increase in NE turnover in MPN and ME that normally occurs during the PM in E_2-treated rats. MPN-DA turnovers were not affected by M but ME-DA turnovers were suppressed during the PM. We next examined the effects of naloxone (NX) during hours when NE turnovers are low (AM) or high (PM) in E_2-treated rats. NX was injected at 0915h or 1215h and blood was collected hourly for 7-9h. When given at 0915h, NX elevated LH 3X (100 to 300 ng/ml) and suppressed PRL serum levels by 1000h. LH returned to control levels by 1500h and PRL values to normal by noon and thereafter both hormones paralleled control surge patterns during the PM. NX, given at 1215h produced a "progesterone like" amplification and advancement of LH release (LH peak values 4000 vs 1000 ng/ml for controls). PRL surge values in NX-treated rats (1215h) were similar to controls. These data suggest that blockade of the opiate system allows more effective expression of NE stimulatory effects on LHRH release. We next examined NX effects in E_2P_4-treated rats (day 3) in which diurnal LH surges and PM NE turnovers are absent and DA turnover is elevated. NX, given at 0915h had no effect on basal LH and only elevated serum LH from 100 to 300 ng/ml if given at 1215h. In contrast, NX still suppressed serum PRL in the AM but had no effect on PM PRL surges in these rats. Finally, we examined the effects of NX in 7 day castrated male rats treated for 2 days with E_2. NX, given at 0900 or 1200h elevated LH from 140 to 240 ng/ml in 15 min but by 60 min LH had returned to baseline. NX caused a concurrent decline in PRL by 15 min with return to baseline by 60 min. Neither LH nor PRL serum levels changed thereafter during the next 4h. Preliminary studies during the AM of day 2 in E_2-treated females indicate that NE turnover does not increase in MPN or ME after NX although DA turnovers increase in ME. Since M increases 5-HT and blocks NE turnover, whereas, NX increases DA turnover, we suggest that the opiate system interacts primarily with the NE system to modulate NE-induced LHRH release. Supported by NIH 02138-22.

273.2 RISE OF BETA-ENDORPHIN SERUM LEVELS IN PRE-MENSTRUAL DISTRESS SYNDROME. A. James Giannini, Robert H. Loiselle, William A. Price. Department of Psychiatry, Northeastern Ohio Universities College of Medicine, Rootstown, OH.

Premenstrual tension syndrome was hypothesized to be the result of beta-endorphin (bE) withdrawal. Sixteen women complaining of symptoms of PMS had bE levels measured on the seventh and twenty-fourth day of each woman's menstrual cycle. A statistically significant decline in bE levels was seen as the cycle progressed ($p < .01$). Symptom severity was inversely proportional to net change in bE levels ($p < .02$). It is hypothesized that the attenuation of bE decline may be a compensatory mechanism to modulate PMS symptom severity.

273.3 TEMPORAL EFFECTS OF MORPHINE ADMINISTRATION ON TUBEROINFUNDIBULAR DOPAMINERGIC NEURONAL ACTIVITY. P. Safier*, J. Rabii, L. Grandison, Dept. Biol. Sci., Rutgers Univ. and Dept. Physiol., UMDNJ, Piscataway, N.J. 08854.

It has recently been reported that morphine administration causes a biphasic effect on the tuberoinfundibular dopamine turnover and release (Gudelsky et al., Endocrine Society Abstracts, p. 165, 1983). We have attempted to characterize the response of the tuberoinfundibular dopaminergic neurons to opiates, with respect to time after a single injection and to repeated injections of morphine sulfate (15 mg/kg, sc). The rate of depletion of median eminence dopamine content, following synthesis inhibition by -methyl-p-tyrosine, was used as an index of dopaminergic neuronal activity. A single dose of morphine produced an inhibition of dopamine turnover at 1 hour, but by 4 hours the turnover rate was higher than control levels. By 8 and 12 hours, turnover rates had returned to control values. In rats given 2 injections of morphine 4 hours apart, the degree of inhibition of dopamine turnover produced by the second injection (29%) was not significantly different from that produced by the initial injection (51%). However, the turnover rate at 1 hour following an initial injection was significantly lower than the rate at 1 hour after a second injection. These results indicate that the response of the tuberoinfundibular dopaminergic neurons to morphine is multiphasic: an initial decrease in dopamine turnover at 1 hour, an increase at 4 hours and a return to normal levels at 8 and 12 hours post injection. The increase in dopamine turnover 4 hours after an initial injection can be attenuated by a second injection of morphine. However, the absolute rate of dopamine turnover following a second injection is similar to the rate in controls but higher than the rate 1 hour after the initial morphine injection. These changes in dopaminergic neuronal activity are inversely related to the secretory rate of prolactin in male rats similarly treated. (Supported in part by NIH grant DA02227, a grant from Charles and Johanna Busch Memorial Fund and a grant from Rutgers University Office of Research and Sponsored Programs.)

273.4 ELEVATED BETA-ENDORPHIN LEVELS IN PREMENSTRUAL TENSION SYNDROME. R. H. Loiselle, A. J. Giannini, S. Kalavsky, M.C. Giannini* and W.A. Price. Department of Psychiatry, Northeastern Ohio Universities College of Medicine, Rootstown, OH.

Fifteen women with diagnosis of premenstrual tension syndrome (PMS) were compared with fifteen female age-matched controls. Cortisol, TRH and beta-endorphin levels were drawn for both groups at days 0, 7, 14, 21 and 28 of each woman's cycle. Changes in cortisol and TRH were nonsignificant. Women with PMS had significantly elevated levels at day 7 ($p < .05$), day 14 ($p < .05$), and day 21 ($p < .02$) levels. Beta-endorphin levels also rose significantly in women with PMS ($<.05$) but not in controls. The rises were viewed as compensatory responses since PMS symptoms are similar to those of opiate withdrawal (Giannini et al 1984, Price et al 1984).

273.5 DURATION OF OPIATE RECEPTOR BLOCKADE DETERMINES THE EXTENT OF BODY AND BRAIN DEVELOPMENT IN RATS: A NEW ROLE FOR ENDOGENOUS OPIOID SYSTEMS. I.S. Zagon and P.J. McLaughlin. Department of Anatomy, The M.S. Hershey Medical Center, Hershey, PA 17033.

Recent experiments have shown that administration of opioid antagonists exert an extraordinary effect on growth. Daily administration of a relatively "high" dosage of naltrexone (NTX) to preweaning rats increases body and organ weights, accelerates brain development, and increases maturation of spontaneous sensory and motor behaviors. Daily injections of a "low" dosage of NTX delays somatic and neurobiological development. An important question in these studies concerns the relationship between growth processes, drug dosage, and the pharmacological properties of NTX (e.g., ability to block the opiate receptor). The present study was undertaken in order to (a) examine the effects of various dosages of NTX on body and brain development, (b) determine the effect of NTX dosage on opiate receptor blockade, and (c) elucidate the relationship between duration of receptor blockade and somatic and neurobiological growth. Sprague-Dawley rat pups injected daily (SC) with 20, 50, or 100 mg/kg NTX from birth to day 21 had increases of 16-22% from control levels in body wieghts at day 21, as well as 6-13% increases in brain weights. These dosages blocked the opiate receptor for the entire 24 hr each day. In contrast, daily injections of 0.1, 1, or 10 mg/kg NTX resulted in young rats with body and brain weights markedly reduced from control levels. These low dosages of NTX only blocked morphine-induced antinociception for 4-12 hr/day. To further investigate whether body and brain development was related to opiate receptor blockade or to drug dosage per se, rats were injected 3 times daily with 3 mg/kg NTX or once daily with 9 mg/kg NTX. At 21 days of age, rats given 3 mg/kg NTX three times daily, a dose equivalent to the total daily injection of 9 mg/kg NTX, and which in effect blocked the opiate receptors for the entire 24 hr period, had increases of 31% and 10% in body and brain weights, respectively, in comparison to control levels. However, rats receiving 9 mg/kg NTX, a dosage that only blocked the opiate receptor for 6-12 hr/day, were retarded in growth by 6%. These results show that the duration of opiate receptor blockade rather than drug dosage is the key element in determining developmental events, and provide evidence that the endogenous opioids play an important role in regulating growth.

273.6 CEREBROSPINAL FLUID FROM CONVULSED RATS CAUSES A NALOXONE-REVERSIBLE INCREASE IN THE SEIZURE THRESHOLD OF RECIPIENT ANIMALS: REGULATION OF POSTSEIZURE INHIBITION BY ENDOGENOUS OPIOID SYSTEMS. J.B. Long and F.C. Tortella. Neuropharm. Br., Dept. of Med. Neurosci., Div. of NP, Walter Reed Army Institute of Research, Washington, D.C. 20307.

Using various animal models of experimental seizure activity, several groups have suggested that seizure-activated endogenous opioid systems play a functional role in the postseizure inhibition of subsequent seizure activity. We have shown that naloxone antagonizes the effect of maximal electroshock (MES) to increase the threshold to subsequent flurothyl seizures (Tortella and Cowan, Life Sci. 31:2225, 1982) and reverses the progressive decrease in seizure severity caused by repeated MES (Tortella et. al. Neurosci. Abst., 1983). Additionally, postictal seizure protection is completely abolished in morphine-tolerant rats (Tortella et. al, Fed. Proc., 1984). The purpose of the present study was to determine if seizures cause an increase in opioid-like, anticonvulsant endogenous material in cerebrospinal fluid (CSF).

To test this idea, the rat flurothyl model was used. CSF was taken from male S.D. rats (300-400 g) at various times (0, 10, 30, 60, and 1440 min) after a single MES-induced seizure. Control CSF was taken from rats identically handled, but not convulsed. The CSF (10 µl) was transferred to the lateral ventricle of untreated recipient rats, and 10 min later the latency (sec ± S.E.M.) to a flurothyl-induced clonic convulsion was recorded in these recipient rats as the seizure threshold. The convulsive threshold to flurothyl following administration of control CSF was 325 ± 7 sec. CSF taken from MES rats caused a maximal significant ($p<0.05$) increase in seizure threshold to 378 ± 18 sec (16% above control) at the time of peak activity (10 min post-MES). Significant anticonvulsant activity was also seen with CSF collected immediately following (0 min) and 30 min post-MES. No residual anticonvulsant activity was noted with CSF collected at 60 min or 1440 min after MES seizures. Naloxone (10 mg/kg, s.c., but not 1 mg/kg, s.c.) administered to the recipient rats 10 min prior to the i.c.v. injection of 10 µl CSF completely antagonized the CSF-induced increase in seizure threshold (325 ± 11 sec).

Using this novel approach, we propose that generalized seizures activate the release into the CSF of opioid-like substances which may function postictally as endogenous anticonvulsants during postseizure inhibition.

273.7 PHARMACOKINETICS AND EFFECTS OF MORPHINE PELLETS IN THE RAT. B.C. Yoburn, T. Huang*, J. Chen*, A. Cohen* and C.E. Inturrisi*, Dept. of Pharmacology, Cornell Univ. Med. Coll., New York, NY 10021.

Subcutaneous implantation of morphine pellets is a standard method of producing tolerance and dependence to opiates in the rat. However, the drug-release characteristics of this procedure and the relationship between morphine pharmacokinetics and effects have not been well-defined. Therefore, we have addressed these issues in the following study.

Male rats each with a chronic arterial cannula were implanted subcutaneously with 2 morphine (75 mg morphine/pellet) or placebo pellets individually wrapped in nylon mesh. At 2, 4, 6, 12, 24, 36, 48, 60 and 72 hrs post-implantation analgesia (tailflick), plasma morphine (PM) levels (extraction and RIA) and body weight were determined. Analgesia onset at 2 hrs, peaked at 4-6 hrs and by 36 hrs was not different ($p>.05$) from control. At 2 hrs mean (±SEM) PM was 196±46 ng/ml and at 4-6 hrs increased to 310±41 ng/ml. By 36 hrs PM declined to 205±29 ng/ml where it remained until 72 hrs when the pellets were removed. Testing (analgesia, PM, weight) continued at 2, 4, 6, 12, 24 and 48 hrs following pellet removal. Tailflick latencies did not differ ($p>.05$) between the groups at these times. Following pellet excision plasma morphine declined biexponentially with a mean $t_{\frac{1}{2}}(\alpha)$ of 0.60 hr and a terminal $t_{\frac{1}{2}}(\beta)$ of 7.3 hr. All animals lost weight following pellet removal. Weight loss for morphine implanted rats was significantly ($p<.05$) different from control by 12 hrs and peaked at 48 hrs (10.1% loss). Total dose of morphine released was determined by assaying (HPLC) the amount of drug remaining in the excised pellets. Over the 72 hr implant the mean (±SD) total dose was 25.1 mg (±2.4).

The results of this study indicated that release of morphine from subcutaneous pellet implants is rapid during the first 6 hrs and then becomes relatively constant. We found that in the rat a significant decline in PM (85%) was a prerequisite for the onset of withdrawal as indexed by body weight loss. Further, we determined that 72 hr plasma morphine levels were significantly ($p<.05$) correlated ($r=+.88$) with peak weight loss. Supported in part by NIDA Grant DA-01457.

273.8 MECHANISM OF MORPHINE WITHDRAWAL IN SPINAL-TRANSECTED RATS. D.C. Marshall and J.J. Buccafusco. Depts. of Pharmacology and Toxicology, and Psychiatry, Medical College of Georgia and V.A. Medical Center, Augusta, GA 30912.

The autonomic component of morphine withdrawal may be assessed by measurement of the increase in mean arterial pressure(MAP) associated with abstinence (Pharmacol. Biochem. Behav. 18:209,1983). It is possible that there may be a significant spinal component to this response since intrathecal administration of naloxone(N) in dependent-intact rats elicits many of the autonomic as well as behavioral signs of withdrawal. The purpose of this study was to determine whether withdrawal induced hypertension also could be elicited in spinal-transected animals. Rats were made physically dependent via chronic infusion of morphine (35-100 mg/kg day) over 5 days. Dependent rats were anesthetized with halothane, artifically respirated and transected at the C1 spinal level. After recovery from anesthesia, N (0.5 mg/kg, i.a.) produced an immediate increase in MAP of 72±3mmHg which gradually declined to 24±4 mmHg above preinjection levels by 60 min. N produced no effect in non-dependent, transected rats. The pressor response to N in spinal, morphine dependent rats was mediated by the sympathetic nervous system since it was blocked with phentolamine(4 mg/kg) or hexamethonium(100 mg/kg) although it was unaffected by adrenalectomy. The site of action appeared to be within the spinal cord itself since the pressor response also was abolished by spinal pithing(C1 to L4). To determine whether local afferent pathways were involved in this response dorsal roots were selectively lesioned surgically, without affecting the doral horn cells or the ventral roots in transected, dependent rats. N was ineffective in eliciting withdrawal hypertension in doral root lesioned rats. The opiate receptors involved were localized within the cord rather than on the dorsal root ganglion since pretreatment of dependent, transected rats(intact roots) with an intrathecal injection of morphine (1200 µg) significantly inhibited the pressor response to N. Equivalent i.a. pretreatment with morphine was without effect. We conclude that 1) a withdrawal syndrome can be elicited through the spinal cord, independent of supraspinal influences, 2) the increase in MAP is mediated via the sympathetic nervous system exclusive of the adrenals and the response arises within the spinal cord and 3) afferent dorsal root input is required for expression of the withdrawal response. Supported by the Medical Research Service of the V.A.

273.9 CONVULSANT-ANTICONVULSANT EFFECTS OF OPIATES. ARE THEY MEDIATED THROUGH ENDOGENOUS OPIOID RECEPTORS? Spillantini,M.G.*, Mele,L., and Massotti, M. Lab. di Farmacologia, Istituto Superiore di Sanità, Roma, Italy.

Convulsant or anticonvulsant effects of opiates are extensively described in the literature. These opposing effects seem to be dependent in part upon the doses and route of administration (Urka and Frenk, Brain Res., 246, 121, 1982).

In rabbits, pretreatment with morphine (0.25 mg/kg iv), cyclazocine (0.05 mg/kg iv) and naloxone (0.02 mg/kg iv) strongly reduced both the incidence and duration of EEG and behavioral seizures due to administration of Na penicillin (50-150 Units) into the sensorimotor cortex. In addition, the effects of both morphine and cyclazocine were not sensitive to the administration of the high doses of naloxone (5-20 mg/kg iv), which per se display a potentiating effect on the seizures due to the low doses of Na penicillin (75 Units).

These data show two paradoxical lines of evidence: i) the demonstration that opiate agonists and antagonists display anticonvulsant effects at very low doses, thus indicating that these effects are mediated through a true occupation of their specific "pharmacological receptors"; ii) despite the assumption that in these experimental conditions morphine and cyclazocine act through occupation of these "receptors", their effects are not naloxone sensitive. These findings, togheter with the data indicating the convulsant effects of opiates at very large doses, seem to confirm the hypothesis of Urka and Frenk (1982), who suggest the existence of two opiate systems: one is excitatory and epileptogenic, sensitiveto the high doses of opiate agonists and to the antagonism by naloxone; another possessing inhibitory and antiepileptogenic properties, sensitive to the low doses of opiate agonists and insensitive to the antagonism by naloxone (see Jaquet, TINS, june, 140, 1979; Marcais et al., Life Sci., 28, 2737, 1981), which on the contrary also displaysan apparent agonistic effect. If so, this could explain the complexity of the regulation by endogenous opioid systems on the convulsant phenomena.

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273.10 ANTIARRHYTHMIC AND ANTIDEPRESSANT EFFECTS OF NALOXONE IN THE ISOLATED PERFUSED RAT HEARTS FOLLOWING MYOCARDIAL ISCHEMIA AND REPERFUSION. C.Y. Zhan*, A.Y.S. Lee* and T.M. Wong* (SPON: P.W.F. Poon). Department of Physiology, University of Hong Kong, Hong Kong.

The effects of naloxone on the electrical activities, left ventricular diastolic and systolic pressures and heart rate following myocardial ischemia and subsequent reperfusion were studied in the Langendorff perfused rat heart model. Pretreatment of naloxone (100 µg) into the isolated perfused rat heart abolished the reduction in left ventricular pressures and heart rate and attenuated greatly the arrhythmias due to myocardial ischemia and reperfusion. Administration of naloxone (200 µg or 600 µg) into the fibrillating heart induced by myocardial ischemia and reperfusion also attenuated the arrhythmia in a dose dependent manner. The results indicate that the endogenous opioid peptides are probably responsible at least partly for the arrhythmias and depressant effects of myocardial ischemia and reperfusion. (Supported by Hong Kong University Research Grant 335/034/0008 and Wing Lung Medical Research Fund 311/030/8009/64 to T.M.W. and China Medical Board Fellowship to C.Y.Z. from Zhongshan Medical College, China).

273.11 STRUCTURE-ACTIVITY STUDIES OF β-CARBOLINE ANALOGS
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Various β-carboline-3-carboxylate esters bind with extremely high affinity to benzodiazepine receptors and inhibit the physiological actions of benzodiazepines. To date, structure-activity studies have been primarily concerned with variations in the ester and related groups at C_3. In order to continue the study of stereochemical requirements for high affinity of this class of benzodiazepine receptor antagonists, we have synthesized and studied receptor affinity and antagonist activity of systematically altered β-carboline analogs. The compounds were chosen for synthesis in order to explore the importance of the N_9-hydrogen, the N_2-aromatic nitrogen, and the C_3-ester moiety for high benzodiazepine receptor affinity and antagonist activity of β-carboline.

Both the unsaturated nitrogen of the aromatic ring and an appropriate group at C_3 appear to be important for high benzodiazepine receptor affinity. An in-plane interaction between the imine N_2 and a cationic recepotr site, rather than stacking interactions, appears to be required for high affinity. The electron-withdrawing properties of the C_3-ester group do not appear to be crucial for high affinity. Rather, another key interaction of the group at C_3, common to high-affinity 3-ester and 3-nitrile analogs but not present in the amide, seems necessary. Thus a specific C_3-group interaction along with key aromatic nitrogen interactions appear essential for high receptor affinity and antagonist activity.

273.12 BRAINSTEM PATHWAYS WHICH MEDIATE CENTRAL CARDIOVASCULAR ACTIONS OF OPIOIDS. A. H. Hassen, G. Feuerstein and A. I. Faden (SPON: B. M. Cox). Neurobiology Research Unit, USUHS, Bethesda, MD 20814.

Hindbrain microinjection of opioids elicited cardiovascular (CV) responses that were receptor- and site-specific in anesthetized, artificially ventilated rats. Mu and mu-delta agonists produced naloxone-sensitive, dose-related increases in mean arterial pressure (MAP) and heart rate (HR) following injection into the N. Tractus Solitarius (NTS) and N. Ambiguus (NA) regions of the hindbrain (Peptides 3: 1031, 1982; Neuropharmacol.24: 407, 1984). Kappa agonists elicited a naloxone-insensitive, dose-related decrease in MAP following NA injection; no MAP changes were observed following NTS injections (J. Neuroscience ,In Press). It has been shown that sympathetic pathways in the spinal cord as well as vagal parasympathetic pathways mediate some opioid responses. The present study was designed to ascertain whether the CV responses to mu and kappa agonists are mediated by the same pathway and if pressor responses and tachycardia are dependent on intact baroreceptor pathways.

Pentobarbital anesthetized, artificially ventilated rats were observed for 15 min before and after a single injection (0.1 µl) into the NTS (Obex, L:0.5mm, V-0.5mm) or NA (1.9 mm rostral to the obex, L:1.9mm, V:-2.3mm) regions of the hindbrain. The mu agonist DAGO (300 pmol) increased MAP and HR following NTS (+35 ± 4 mmHg, +54 ± 4 BPM, n=5) or NA (+16 ± 4 mmHg, +33 ±7 BPM, n=6) injections in intact rats; no cardiovascular responses were observed following DAGO injection into either region following complete C1 spinal transection. NA injection of the kappa agonist MRZ (16 pmol) lowered the MAP in intact animals (+23 ± 4 mmHg, n=6) and in transected animals (-17 ± 4mmHg, n=5). However, when transected animals were vagotomized (n=4) no change in MAP was observed following MRZ injection. Vagotomy alone did not attentuate the depressor response (-39 ±12 mmHg, n=6). Animals which lacked baroreceptor reflex activity 10 days after central deafferentation still increased MAP (+22 ± 9mmHg) and HR (+25 ± 10 BPM) following DAGO injections (n=4). It is concluded that the CV responses to mu and kappa agonists are mediated by different CNS pathways and are not dependent on intact baroreceptor activity.
Supported by USUHS protocol RO 9201.

EXTRASTRIATE VISUAL AREAS

274.1 BOUNDARIES BETWEEN EXTRASTRIATE VISUAL CORTICAL AREAS IN THE CAT: THEIR LOCALIZATION AND VARIABILITY. H. Sherk, Dept. of Biological Structure, U. of Washington, Seattle, WA

A major problem in the investigation of extrastriate visual cortical areas is the difficulty in determining their boundaries histologically. This is particularly troublesome because of the possibility that the locations of such boundaries vary between individuals, as is the case for auditory cortex (Merzenich et al., 1975). The experiments reported here suggest a simple method for marking the boundaries between a number of visual areas in one animal.

Many visual cortical areas abut each other along their representations of the area centralis (AC) (Tusa et al., 1981); thus an injection of tracer that labels these AC representations should mark the boundaries between several pairs of visual areas. I therefore injected 3H-leu and 3H-pro into the AC representation of area 19 (located electrophysiologically) in the right hemispheres of 7 cats, and examined the resulting pattern of projection to the left hemisphere autoradiographically.

These experiments defined portions of the borders between 4 pairs of visual areas: areas 17 and 18, areas 19 and 21a, the Clare-Bishop area and PLLS, and areas 20a and 20b[1]. In addition, discrete patches of label were present in area VLS, the crown of anterior area 7, and the suprasplenial visual area.

The locations of boundaries between most areas varied from cat to cat. One striking instance was the border between the Clare-Bishop area and PLLS: in some cats this was situated close to the bottom of the suprasylvian sulcus, in others it was placed well up its lateral bank, so that the AC representation of PLLS extended out onto the posterior ectosylvian gyrus. The boundary between areas 19 and 21a was also variable, and in many cats this representation of the AC was split into a posterior and an anterior segment, with more peripheral visual field intervening between them. I have confirmed this split in area 19 both by physiological recording and by tracer injections into the AC representation of the lateral geniculate nucleus.

These results indicate that the locations of boundaries between extrastriate cortical areas vary between individuals, but can be demarcated at least partially in any particular cat by a single injection of tracer.

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[1]Abbreviations refer to the nomenclature of Tusa et al.(1981)

274.2 ASPARTATE AND GLUTAMATE AS SYNAPTIC TRANSMITTERS OF PARALLEL VISUAL CORTICAL PATHWAYS. T.P.Hicks, W.D. Ruwe, W.L.Veale, and J.C.Veenhuizen. Dept. of Medical Physiology, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada. T2N 4N1.

The lateral suprasylvian area (LSA) of the cat's visual cortex consists of a number of distinct regions, each containing a representation of the visual field. Two such areas, the PLLS and PMLS, have been shown to possess cells which may be synaptically activated at short latencies by electrical stimulation of both the 17/18 border region and the homotopic, contralateral visual areas of the LSA. These cortico-cortical synaptic activations of single cells are blocked by pharmacologically specific antagonists of NMDA receptors, suggesting that glutamate and/or aspartate may be synaptic transmitters within the LSA. We report here the results of an in vivo investigation which examined the release of endogenous excitatory amino acids evoked from the PMLS and PLLS by stimulation of the contralateral LSA and ipsilateral 17/18 border region.

Cats were anaesthetized with sodium pentobarbital (35 mg/kg, i.p.). Electrical stimuli were delivered through concentric bipolar electrodes (2-20 V., 0.2-0.5 msec pulse duration, 200-300 Hz for 300-500 msec). Push-pull cannulae were lowered into PMLS or PLLS through a previously implanted stainless steel guide tube. The LSA was perfused with an artificial CSF containing 5 mM glucose and occasionally having elevated levels of K^+, or high Mg^{++}/low Ca^{++}. Perfusates obtained from sites in PLLS contained elevated levels of glutamate (as determined by HPLC with fluorescence detection) when stimuli were delivered to the ipsilateral, primary visual cortex. By contrast, when sites in PMLS were perfused during ipsilateral stimulation sequences, aspartate release always exceeded that of glutamate. Perfusion of the fundus of the posterior LSA elicited either a greater relative release of glutamate, or approximately equal levels of both substances. High K^+ in the perfusion medium enhanced resting release while high Mg^{++}/low Ca^{++} reduced or abolished the stimulation-evoked release. Contralateral LSA stimulation evoked glutamate release from PMLS in one instance. These data support the contention that excitatory amino acids are cortico-cortical transmitters of visual pathways. They extend this view further by linking different synaptic transmitters with distinct parallel visual pathways, which are also segregated anatomically and functionally.
(This work was supported by the MRC of Canada and the Alberta Heritage Foundation for Medical Research).

274.3 A THALAMO-CORTICAL SUBSYSTEM IN THE CAT FOR THE DETECTION OF EXPANDING VISUAL FLOW FIELDS OF MOTION. J.P. Rauschecker, A. Friederichs*, M.W. von Grünau*, C. Poulin*. (SPON: European Neuroscience Association). MPI für biologische Kybernetik, D-7400 Tübingen, FRG.

In the cerebral cortex multiple representations of the visual field exist in a partly hierarchial scheme. In addition, parallel inputs are provided by various thalamic nuclei. Anatomical tracer studies have recently yielded strong support for the classical notion that a particularly strong reciprocal connection exists between a particular cortical area and one principal thalamic relay nucleus. Thus, like area 17 being intimately connected with the lateral geniculate nucleus, areas PMLS and PLLS of the cat's lateral suprasylvian visual cortex seem to be tied up with the lateral and medial parts of the lateral posterior nucleus of thalamus (LP_l and LP_m), respectively. We have analysed single unit responses in PMLS and LP_l under visual and electrical stimulation, in order to see whether the reciprocal connection between both levels results in similar response properties. Recording sites were verified histologically; in particular, LP_l was identified from ACh-esterase stained sections (Graybiel and Berson, 1980).

We have found that PMLS and LP_l share indeed a striking number of features in their single unit response properties. Many units display pronounced binocular facilitation. Velocity tuning is broad reaching from 1 deg/sec up to 500 deg/s and more in a single cell. Spatial frequency tuning is also broad, high frequency information being preserved despite large receptive fields. The majority of cells are selective for the direction of a moving spot of light. Most significantly, both in PMLS and LP_l a large proportion of cells responds best to movement away from the area centralis into the visual field periphery. Thus, this thalamo-cortical system is maximally activated by optical motion flow-fields expanding radially arround the center of fixation. This could assign a role in visual guidance during forward locomotion to this visual subsystem. Even more generally, one can argue that not a certain cortical area, but a certain thalamo-cortical subsystem may subserve a specific functional role.

274.4 NEURONAL UPTAKE AND LAMINAR DISTRIBUTION OF TRITIATED ASPARTATE, GLUTAMATE, GABA AND GLYCINE IN THE PRESTRIATE CORTEX OF SQUIRREL MONKEYS: CORRELATION WITH LEVELS OF CYTOCHROME OXIDASE ACTIVITY AND THEIR UPTAKE IN AREA 17. E.W. Carroll* and M. Wong-Riley (SPON: R.L. Curtis). Dept. of Anat., Med. Coll. of Wis., Milwaukee, WI 53226.

The uptake of two putative excitatory neurotransmitters (ASP and GLU), and two putative inhibitory transmitters (GABA and GLY), was compared in area 18. We sought to determine if any correlation existed between their uptake and the cytochrome oxidase-rich (C.O.) puffs in laminae II-III as we found for GABA and GLY in area 17. Concentrations of these injected ^{3}H-amino acids (and ^{3}H-leucine controls), ranged from 2.5-6 µCi/0.1-0.2 µl/site. Perfused brain tissues were reacted for C.O., then processed for autoradiography. As in area 17, ^{3}H-labeled C.O.-reactive (C.O./ ^{3}H+) and unreactive (^{3}H+) neurons were found in all laminae with each amino acid tested. The C.O./ ^{3}H+ neurons were, on the average, larger than the ^{3}H+ labeled ones. Few glial cells were labeled. ASP/GLU: Few C.O./ ^{3}H+ or ^{3}H+ neurons were present in II-III. In IV-VI, an increase in both types of labeled neurons was observed, with the majority found in V and VI. Labeled neurons included fusiform (up to 10x19µm), stellate (8-15µm in dia.) and pyramidal-shaped neurons (17x25µm). The C.O./^{3}H+, GLU-labeled stellate neurons in V were slightly larger than similarly positioned, ASP-labeled ones. Fewer ASP-labeled, fusiform-shaped neurons were found than with GLU. The results otherwise were similar to area 17. In contrast to ASP and GLU, GABA and GLY labeled neurons (C.O./^{3}H+ and ^{3}H+) were, as in area 17 more prevalent in II-III. GABA: Labeled neurons (C.O./^{3}H+ and ^{3}H+) were more abundant in II than III, and both types increased in number in IV-VI, with IV less densly labeled. GABA-labeled neurons were often observed for considerable distances (750µm) from the injection sites. Labeled cells included stellate (8-19µm) and fusiform-shaped neurons (9x14µm). No labeled pyramidal cells were observed. Glycine: C.O./^{3}H+ and ^{3}H+ neurons were more common in all laminae when contrasted with the other putative transmitters. Fewer labeled neurons were observed in IV in comparison to III, V and VI. Labeled neurons included stellate (11-19µm), fusiform (13-19µm) and pyramidal cells (19-26µm). The majority of the larger GLY-labeled neurons were found in III. Our preliminary analysis suggests that, as in area 17, more C.O./^{3}H+ neurons (both GABA and GLY) are found within the puffs as opposed to the interpuff regions. (Supported by NIH NS 18121).

274.5 INTRACORTICAL AND INTERHEMISPHERIC MECHANISMS OF VISUAL DISCRIMINATION IN CAT-A CROSS LESION STUDY. K.J. Kaufman. Dept. of Anatomy & Physiology, Univ. of Penna., Phila.,PA 19104.

To examine the flow of visual information between classical and more recently described visual cortical areas in the cat, an experiment was designed to demonstrate in this species, whether discrimination of pattern and form relied on a serial cortico-cortical process, similar to that present in monkey. Asymmetric lesions to areas 17,18,19 on one side, and lateral suprasylvian areas (LSA), 20,21 and 7 on the other were followed by callosum transection. Such extensive, asymmetric lesions, left visual learning unaffected except for a mild retention deficit in difficult form discriminations. Subsequent transection of the corpus callosum in six animals resulted in absolutely no impairment in retention for any of the learned stimuli. Thus the cat differs from the macaque in that these two parts of visual cortex do not need interhemispheric connections for visual discrimination.

Subsequent section of left or right optic tract limited the visual input to areas 17,18,19 on one side, or to the remaining visual areas on the other. When visual function was mediated by areas 17,18,19 perfect retention of all discriminations was found. When these areas were deprived of visual input and behavior was dependent on areas 7,20,21,LSA and part of 19, retention was absent and only certain simple discrinination tasks were relearned.

Areas 17,18, and 19 on one side of the brain-when functioning isolated from any other retinotopic cortical visual area, including the mirror symmetric regions of the opposite hemisphere-could handle the most complex discrimination tasks. Pattern and form discrimination did not require serial processing by cortico-cortical connections beyond area 19 in the cat.

Summary of Findings and Conclusions: 1) a small and specific deficit in the performance of complex form discriminations was observed after cortical lesions to areas 17,18,19 on one side; to 20,21,7 and LSA on the other. Several ipsilateral cortico-cortical connections were probably responsible; 2) cats with asymmetric cortical lesions, and no invasion of white matter, clearly showed that, unlike comparable areas in monkey, areas 17,18,19 and 20,21,7 and LSA did not rely on callosal connection; in cats with visually isolated areas 20,21,7,LSA-these cortical areas mediated retention of flux, simple form discriminations learned as normals but these areas were not able to make functional memory traces for new discriminations in extrastriate areas.

274.6 MORPHOLOGY OF CALLOSAL CELLS IN AREA 19 AND LATERAL SUPRASYLVIAN AREAS IN CAT VISUAL CORTEX. A. Naporn, N. Berman and B.R. Payne, Med Coll of Penn, Philadelphia PA

Homotopic callosal connections between areas 17 and 18 originate from cells which are distributed across a number of cortical laminae and which are of a variety of morphological types. However, pyramidal cells are the most common cell type and they are located predominantly in laminae II and III. To determine whether the cells that form the origin of heterotopic callosal projections from non-primary visual areas to areas 17 and 18 show a similar morphology and laminar distribution, horseradish peroxidase was injected into areas 17 and 18 of one hemisphere. Cells were visualized by reacting alternate coronal sections with either tetramethylbenzidine or diaminobenzidine and hydrogen peroxide. Significant numbers of callosal cells containing reaction product were in areas 19, AMLS, PMLS, and PLLS of one hemisphere.

Areas located progressively further from primary visual cortex show a gradual change in laminar distribution of callosal cells such that an increasingly larger fraction of these cells are in laminae V and VI; and there is a decreased frequency of pyramidal and stellate cells and an increased frequency of triangular, fusiform and multiform cells (classification scheme of Cajal, 1911). In area 19, pyramidal cells are the major cell type and are primarily in laminae III and IV. Stellate cells are the second most common cell type and are also in laminae III and IV. Fusiform and multiform cells are occasionally located in lamina VI. In PMLS and AMLS callosal cells are divided almost equally between supragranular and infragranular layers. Pyramidal cells are the only cell type in lamina III and are occasionally located in V and VI. Triangular, fusiform, and multiform cells constitute the majority of cells in lamina VI. Fewer cells overall are in PLLS, of these most are in lamina VI and are predominantly in the multiform category.

Cells in area 19 and in lateral suprasylvian areas which form the origin of heterotopic projections to contralateral primary visual areas 17 and 18 are more frequently located in infragranular layers, particularly lamina VI, than callosal cells in areas 17 and 18 which form homotopic connections. Because the cells in supragranular layers are primarily pyramidal cells, an increase in the proportion of callosal cells in lamina VI is accompanied by an increase in the diversity of morphological types. This diversity suggests that heterotopic callosal projections to areas 17 and 18 have a different functional role than homotopic projections.

274.7 LOCAL CEREBRAL GLUCOSE UTILIZATION IN CORTEX OF AWAKE, BEHAVING CAT IN RESPONSE TO AN OPTOKINETIC STIMULUS. S.J. Herdman and R.J. Tusa, Univ. of Maryland and Johns Hopkins University, Baltimore, MD 21201

The autoradiographic [^{14}C] deoxyglucose technique was used to determine rates of glucose utilization in visually responsive cortex to an optokinetic stimulus in cats. Eye movements were measured with the magnetic field search coil technique. Local rates of glucose utilization (LCGU) were determined with the autoradiographic deoxyglucose method (Sokoloff, L. et al. J. Neurochem 28:899-916, 1977). Control cats (N=2) faced a full-field, stationary, black and white random dot pattern. These cats had spontaneous saccadic eye movements in all directions. The experimental cat was stimulated with the full-field random dot pattern which was rotated around the cat's head in one direction. This stimulus produced optokinetic nystagmus (OKN). Rates of LCGU were determined in 13 cortical areas ipsilateral to the direction of the slow phase movements in the experimental cat and in the corresponding hemispheres of the control cats.

Preliminary findings indicate that there are marked increases in LCGU in areas 17 and 18 in the experimental cat compared with the controls. Unilateral ablation of 17 and 18 does not produce a deficit in the optokinetic response. (Tusa, RJ et al, Neurosci. 9:154, 1983). The results of this study, therefore, suggest that areas 17 and 18 may be responding to the visual stimulus or to the eye movements but are not directly involved in the generation of an OKN response.

Smaller increases in LCGU were noted in areas 21a, 21b, PMLS, and VLS. Areas 21a, 21b, and PMLS have been shown to project to the dorsal terminal nucleus (DTN) which is part of the sub-cortical pathway which generates slow phase eye movements. Another area, AMLS, also projects to DTN but it did not show an increase in LCGU to the moving stimulus. It is not known whether VLS projects to DTN but the increase in LCGU in response to the moving random dot pattern suggests that it may have a role in the generation of an OKN. It has been shown that a unilateral lesion involving areas 21a, 21b, PMLS and AMLS impairs OKN toward the side of the lesion (Tusa, RJ et al, Neurosci. 9:154, 1983).

Other visually responsive areas including 19, 20a, 20b, PLLS, ALLS, and DLS showed no apparent increase in LCGU to the moving dot stimulus. The results of the autoradiographic deoxyglucose study will serve as a guide for further lesion studies to clarify the roles of visually responsive cortex in the generation of OKN. Supported by Found. for P.T.

274.8 CONNECTIONS OF STRIATE CORTEX PROJECTION ZONE, AREA TD, IN TREE SHREWS. M. A. Sesma, V. A. Casagrande, and J. H. Kaas, Departments of Psychology and Anatomy, Vanderbilt University, Nashville, TN 37240.

In an earlier study of visual cortex in tree shrews, we defined four extrastriate regions by connections with striate cortex: Area 18, a temporal dorsal area (TD) and two divisions of temporal posterior cortex (TPd, TPv), and a connection with posterior limbic cortex. Based upon relations with Area 17, TD could represent the homologue of the primate visual area MT. To investigate this further, we traced connections following single injections of WGA-HRP into TD in 3 tree shrews. Within Area TD each injection revealed a broad pattern of intrinsic connections which radiated from the injection site and formed multiple, nearby foci. Results also revealed reciprocal ipsilateral extrinsic connections with all cortical areas that receive direct Area 17 input, as well as with two additional temporal zones, one immediately rostral to TD and one rostral to TPd, and a posterior parietal area rostral and medial to TD. The densest connections were with the posterior parietal area. In addition, TD exhibited callosal connections with Area 18, Area TD, posterior parietal and posterior limbic cortex. The laminar distribution of connections between TD and these areas varied. In Area 17 labeled terminals were found in layer I and cells of origin mainly in supragranular layers. In Area 18, labeled terminals were located in supragranular layers and cells of origin were found in both supra- and infragranular layers. In other regions labeled cells were in both supra- and infragranular layers while terminals were distributed through the supragranular and granular layers.

The connections of TD and striate cortex in tree shrews suggest the existence of at least seven extrastriate visual areas, and demonstrate direct visual projections to limbic cortex. The connection pattern of TD is largely consistent with the hypothesis that TD is a homologue of MT in primates. As with MT in primates, TD in tree shrews interacts reciprocally with Area 17 and a number of extrastriate projection zones including one in parietal cortex. However, more definitive conclusions may be possible after studying other aspects of TD, including subcortical connections.

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274.9 FUNCTIONAL CHARACTERISTICS OF STRIATE CORTICAL NEURONS PROJECTING TO MT IN THE MACAQUE. J.A. Movshon and W.T. Newsome. Dept. Psychol., NYU, New York, NY 10003 and Lab. Sensorimotor Res., NEI, NIH, Bethesda, MD 20205.

We examined the properties of neurons in the striate cortex that could be activated by electrical stimulation of MT, an extrastriate visual area thought to be concerned with the analysis of visual motion. After placing a bipolar stimulating electrode in a visuotopically identified region of MT, we made electrode penetrations through all layers of the corresponding region of striate cortex (V1).

When the stimulating and recording electrodes were properly aligned, we were able to activate about one-tenth of V1 neurons by stimulation of MT. About one-third of these could be shown to be antidromically activated by a collision test; all these cells had very short latencies (1.1-1.7 mS), and were located in layer IVb or near the border between layers V and VI. Most of the remaining electrically-driven neurons were located in layer II, and were orthodromically driven with long latencies (8-85 mS); this may reflect activation by collaterals of other neurons projecting to MT.

All of the antidromically-driven neurons we found were of the special complex type. They were binocularly activated, orientation selective and (with one exception) direction selective. They had unusually large receptive fields for V1, but nonetheless tended to prefer moderately high spatial frequencies and to respond briskly to short stimuli. They were relatively poorly tuned for orientation and spatial frequency. They often had strikingly good temporal resolution and could respond to frequencies as high as 50 Hz. We did not observe any sensitivity to the color of the stimulus. In all these respects, V1 neurons projecting to MT generally resemble MT neurons themselves.

MT, however, contains neurons that are "pattern direction selective", responding to the direction of motion of stimuli without regard to their constituent orientations (Gizzi et al ARVO 1983). All of the V1 neurons projecting to MT, like other V1 neurons, were "component direction selective", responding to the motion of oriented contours rather than of whole patterns. Thus the major qualitative difference between MT and its input from V1 is a sensitivity to the global motion of complex visual patterns.

274.10 **CORTICAL CONNECTIONS OF AREA V3 IN MACAQUE EXTRASTRIATE CORTEX.** D.J. Felleman and D.C. Van Essen, Division of Biology, Caltech, Pasadena, CA 91125.

Previous work from this laboratory has shown that the cortex immediately anterior to V2 is characterized by several dorso-ventral asymmetries which include: 1) connections with V1, 2) patterns of interhemispheric connections, 3) myeloarchitecture, and 4) neuronal response properties. Taken together these data strongly suggest that the strips of cortex anterior to dorsal and ventral V2 should be considered separate visual areas, with the dorsal strip termed V3 and the ventral strip termed the ventral posterior area, VP.

We report here on the cortical connections of V3 as revealed by injections of ^{3}H-proline and/or HRP into physiologically identified sites in 4 hemispheres. Myeloarchitecture was used to define the locations and extents of areas V3 and MT. In 3 cases the corpus callosum was cut and the resultant degeneration was used to delimit certain visual areas. The laminar patterns of HRP filled cells and autoradiographic label was used to determine the hierarchical relationships of V3 to other visual cortical areas.

One unexpected result was that the projection from V1 to V3 originates almost exclusively from layer 4B. V3 receives only one other ascending input, from the superficial layers of dorsal V2. Feedback from V3 terminates in layers 4, 1, and 6 of V1 and 1-3 and 6 of V2. Both the feedfoward and feedback connections of V1 and V2 with V3 were usually located in discrete patches.

V3 was found to provide consistent ascending input to 3 other visual cortical areas: V3A, V4, and MT. In one case, feedfoward inputs were also observed to areas MST and POa. Reciprocal feedback to V3 from these areas originated primarily from layers 5 and 6.

The present V3 results, when contrasted with previous results on VP indicate that both areas project to V3A, V4, MT, and perhaps MST. V3 has strong connections with V1 which are missing in VP, whereas VP projects to area TF and to a region of the dorsal prelunate gyrus. These differences further substantiate the distinction of these two visual areas. Furthermore, these results indicate that layer 4B of V1 provides parallel outputs to several extrastriate visual areas which may be involved in different aspects of information processing.

Supported by NIH grant EY02091.

274.11 THE ROLE OF EYE POSITION ON THE VISUAL RESPONSE OF NEURONS IN AREA 7A. R.A. Andersen, G.K. Essick* and R.M. Siegel. The Salk Institute, La Jolla, CA 92037.

In previous experiments (Andersen and Mountcastle, 1983) we reported that the angle-of-gaze strongly modifies the visual sensitivity of neurons in area 7a of macaque monkeys. Control experiments indicated that an extra-retinal eye position signal was responsible for this modification in the visual response. We have now examined this angle-of-gaze effect in detail to determine whether it results in the coding of visual stimuli in head coordinates. The receptive fields of area 7 neurons were first mapped using small flashes of light. The receptive fields were large (50° to 80° in diameter), bilateral, and generally gave excitatory on-responses across the entire receptive field. Stimuli were then presented at the same retinotopic locations in the center of the receptive fields, but with the animal fixating (with head fixed) nine different locations within a 40 by 40 degree grid on a tangent screen. Of 113 cells from 3 hemispheres tested in this manner 81 showed a strong change in the visual response dependent on gaze angle. One quarter of these cells were tested in both light and complete darkness and in all cases no change in the effect was observed. We completely remapped the receptive fields of 24 neurons at their least and most responsive gaze angles. In 18 of these cells the receptive fields remained retinotopic and only the magnitude of the response changed with eye position. In another 6 cells the visual response was completely absent at the non-preferred direction of gaze. For 7 of these 24 cells we performed another test in which the stimulus was always presented at the same position on the screen, defined by the best gaze angle and the center of the receptive field, and the animal fixated 13 different locations within a 40 by 40 degree grid. In every case we found that the cells responded differently depending on the gaze angle and thus they were not responding in head coordinates. These experiments indicate that although an extra-retinal eye position signal strongly modifies the response of most light sensitive neurons in area 7a, the transformation to head coordinates can only be considered to be partial. Such a transformation could be achieved by con-verging onto a single cell neurons with different preferred gaze angles and receptive fields whose combinations always resulted in responses which were best for a particular location in head coordinates. Such cells may exist in 7a, but we have not yet recorded from them, or they may be found at another location in the brain. Alternatively, since the information is already present in the response of groups of neurons in 7a, a convergence to single cells may not be necessary for encoding head coordinate locations.

274.12 **NEURONS PROJECTING TO MT AND V4 FROM MACAQUE V2 ARE SEGREGATED INTO DISCRETE STRIPE-LIKE PATCHES.** E. A. DeYoe and D. C. Van Essen. Caltech, Div. of Biology 216-76, Pasadena, CA 91125.

Previous retrograde tracer studies have shown that neurons projecting to visual areas MT and V4 from V2 reside in patches confined predominantly to lamina 3. We sought to determine the relationships among 1) patches projecting to V4 and MT, 2) cytochrome oxidase rich stripes and 3) clusters of physiologically distinct cells within V2. Two fluorescent tracers were used to retrogradely label cells within a region of V2 which also was examined with single and multiple unit recordings. In each of two macaque monkeys, bisbenzimide (BB) was injected at one site in MT and nuclear yellow (NY) was injected at three sites 1-1.5 mm apart in V4. The retinotopic locations of the injection sites were matched to those observed during recordings in V2.

In the best case, blue fluorescent cells labelled from MT and yellow fluorescent cells labelled from V4 were found in discrete, non-overlapping patches mainly in lamina 3 but occasionally in the underlying infragranular layers as well. When reconstructed on a 2 dimensional cortical map, these patches formed alternating labelled stripes separated by unlabelled stripes running nearly perpendicular to the V1/V2 border. The width of a complete sequence, a NY labelled (V4 projection) stripe, unlabelled stripe, BB labelled (MT projection) stripe, unlabelled stripe) was 3-4 mm. The V4 projection stripes were 1-2 mm. wide, the thinner MT projection stripes were 0.6-1.3 mm. wide. At least some of the V4 projection stripes were centered on but were significantly wider than the thin cytochrome oxidase dense stripes. The relationship of the MT projection stripes to the cytochrome oxidase patches remains uncertain.

Single and multiple unit recordings within the region of labelling were suggestive of a greater incidence of wavelength selectivity and lower incidence of directional selectivity in V4 projection patches, with the reverse being true within MT projection patches.

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ACETYLCHOLINE RECEPTORS: GENERAL TOPICS

275.1 ^{3}H-ACETYLCHOLINE (^{3}H-ACh) BINDING TO M-2 MUSCARINIC RECEPTORS IN BRAIN AND PERIPHERAL TISSUES. K.J. Kellar, A.M. Martino*, R.D. Schwartz and D.P. Hall, Jr.*. Georgetown Univ. Schools of Med. and Dent., Washington, DC 20007.

We have developed an assay procedure to measure ^{3}H-ACh (80 Ci/mmol) binding to muscarinic receptor recognition sites. The assay was carried out at 25°C for 60 min in the presence of DFP to inhibit cholinesterases and cytisin to occupy nicotinic receptors. Nonspecific binding was defined in the presence of 1.5 uM atropine. In this assay, ^{3}H-ACh bound rapidly, reversibly and with high affinity to sites with characteristics of an M-2 subtype of muscarinic cholinergic receptor in both brain and peripheral tissues.

Specific binding of ^{3}H-ACh in 8 areas of brain and in the heart atrium was saturable over a concentration range of 2-200 nM. Hill coefficients were close to 1 (0.96-1.04) in all 9 of the tissues examined, indicating that over this concentration range ^{3}H-ACh was binding to a single class of sites in each of the tissues. The equilibrium K_D in most of the tissues was between 20 and 35 nM, but it was consistently higher (60-65 nM) in the hippocampus and striatum. The brain areas with the highest densities of ^{3}H-ACh binding sites were the pons, medulla and cerebral cortex. The lowest density was found in the cerebellum. The number of ^{3}H-ACh binding sites represented 15-25% of the ^{3}H-QNB binding sites in the striatum, hippocampus and cerebral cortex; 50-70% of the ^{3}H-QNB sites in the hypothalamus and thalamus; and 80-100% of the ^{3}H-QNB sites in the cerebellum, pons, medulla and atrium.

In drug competition studies, both muscarinic agonists and antagonists were potent inhibitors of binding. For agonists the range of IC_{50} values was 17-1000 nM and the order of potency was: oxotremorine > ACh > carbachol = methacholine = arecoline > muscarine = pilocarpine > bethanechol >> choline. The antagonists (-)QNB, atropine, scopolamine and dexetimide all had IC_{50} values of 1-7 nM. Levetimide, the inactive enantiomer of dexetimide, was 2000 times less potent, indicating a high degree of stereospecificity of the site. Pirenzepine, a muscarinic antagonist which is relatively selective for the M-1 subtype of receptor, had relatively low affinity for the ^{3}H-ACh site in brain (IC_{50} 850 nM). Neither nicotinic drugs, cholinesterase inhibitors nor hemicholinium-3 competed effectively for the ^{3}H-ACh binding sites.

The binding of ^{3}H-ACh is sensitive to guanyl nucleotides, with the greatest reduction in binding occurring in the atrium and in those brain areas with the largest percentage of M-2 sites. The site is subject to in vivo regulation, and is down-regulated by chronic inhibition of cholinesterase.

275.2 CHARACTERIZATION OF M1 and M2 MUSCARINIC RECEPTOR SUBTYPES IN MURINE NEUROBLASTOMA CLONE N1E-115 CELLS. M. McKinney, S. Stenstrom, and E. Richelson. Mayo Foundation, Rochester, MN 55905.

Muscarinic receptors of clone N1E-115 mediate both cyclic GMP stimulation and the inhibition of PGE_1-stimulated cyclic AMP. The effect on cyclic GMP levels has an EC-50 for carbachol in the range of 18-100 μM, while the effect on cyclic AMP levels has an EC-50 in the range of 0.8-4.0 μM. We studied the action of the antagonists atropine and pirenzepine and performed direct binding studies with [^{3}H]quinuclidinyl-benzilate and [^{3}H]N-methylscopolamine in competition with carbachol or pirenzepine in order to determine whether a single site mediates both responses or whether two separate sites are coupled to the different responses. Atropine inhibited both responses with the same inhibition constant (0.2 nM) as determined by the dose-ratio method, while pirenzepine inhibited the two responses with different inhibition constants (11 nM for the cyclic GMP response and 190 nM for the cyclic AMP inhibition). We classify the cyclic GMP response of N1E-115 cells as "M1" and the inhibitory effect of carbachol on PGE_1-stimulated cyclic AMP as an "M2" response. Complementary binding experiments showed that two binding sites for carbachol were present with dissociation constants of 0.9 μM and 20 μM, in close agreement with the EC-50 values. We have studied the ability of 10 agonists to mediate these two responses and have found that the rank orders by both potency and efficacy were different. Six of the agonists were as effective as acetylcholine at the M2 response, while only two were as effective as acetylcholine at the M1 response. McN-A-343, pilocarpine, and bethanechol were partial agonists for the M2 response (40-70% of acetylcholine), but were much less able to stimulate the M1 response. Notably, oxotremorine was as potent (0.4 μM) and efffective as acetylcholine at the M2 response, but was a very poor agonist at the M1 response, having a cyclic GMP response only in the millimolar range. Additionally, we have found, with sequential subculturing of N1E-115 cells in medium supplemented with newborn bovine serum, a loss of the M1 response and the low-affinity binding site without a reduction of the M2 response or the ability of bradykinin or histamine to stimulate cyclic GMP formation. We conclude that two cyclic nucleotide responses of N1E-115 cells are mediated via separate muscarinic receptor subtypes and that these receptors have different structure-activity relationships and regulatory features. (Supported by Mayo Foundation, USPH Grant MH27692, and AM07147).

275.3 EVIDENCE FOR HETEROGENEITY OF MUSCARINIC RECEPTORS IN RAT SUPERIOR CERVICAL GANGLIA. Paul M. Epstein, Linda J. Ojamaa* and Linda F. Quenzer*. Dept. of Pharmacology, Univ. of Connecticut Health Center, Farmington, CT 06032.

A recent study suggests possible differential coupling of muscarinic receptor subtypes to phosphatidylinositol (PI) and cGMP responses in rat sympathetic ganglia (Patterson and Volle, J. Aut. Nerv. Sys. 10:69, 1984). In this study bethanechol (Bch) promoted both PI turnover and cGMP accumulation; whereas, 4 aminopyridine (4 AP) selectively stimulated PI turnover with no effect on cGMP levels in denervated ganglia. All of these responses were blocked by atropine, suggesting mediation by muscarinic receptors. To further investigate the possibility of heterogeneity of muscarinic receptors in ganglia, we performed binding experiments with [^{3}H] quinuclidinyl benzilate ([^{3}H]QNB), and analyzed drug displacement of this binding. The binding of [^{3}H]QNB to rat superior cervical ganglia homogenates was saturable with ≃ 97% specific binding. Kinetic analysis of binding yielded K_{+1} = 0.70 ± 0.30 x 10^{-9} M^{-1} min^{-1} and K_{-1} = 0.0072 min^{-1}, giving a kinetic K_D = 12.7 ± 6.9 pM (S.D., N = 7). Scatchard analysis of equilibrium binding yielded a K_D = 74.3 ± 16.0 pM and a B_{max} = 568 ± 59 fmol/mg protein (S.E., N = 7). In the presence of 50 pM [^{3}H]QNB, complete displacement of binding occurred with 4 AP, calcium channel antagonists, and known agonists and antagonists of acetylcholine receptors. The K_i's and Hill coefficients (n_H) derived from these displacement curves are shown below:

DRUG	$K_{i\,app}$	n_H	DRUG	$K_{i\,app}$	n_H
atropine	0.51nM	0.87	gallamine	1.9μM	0.66*
pirenzepine	20 nM	0.50*	DMPP[a]	3.4μM	0.75*
oxotremorine	0.55μM	0.72*	bepridil	0.9μM	1.25*
McN-A-343	4.3 μM	0.77	nicardipine	1.5μM	0.97
carbachol	13 μM	0.48*	verapamil	4.5μM	1.06
bethanechol	55 μM	0.60*	4 AP	120 μM	0.93

*Significantly different from one, $p < 0.05$, N = 3.

[a]DMPP = dimethylphenylpiperazinium.

It is noteworthy that the Hill coefficient for 4 AP displacement is close to one, whereas that for common muscarinic agonists and the antagonist, pirenzepine, are significantly less than one. This is consistent with the observation of Patterson and Volle that 4 AP may recognize one type of muscarinic receptor, whereas Bch may recognize more than one type. Supported by USPHS NS07540 and Miles Laboratories.

275.4 CYSTEINE RESIDUES CONTRIBUTING TO THE BINDING SITE DISULFIDE BOND IN THE NICOTINIC ACETYLCHOLINE RECEPTOR. P.N. Kao*, A.J. Dwork*, R.-R. Kaldany*, M.L. Silver*, J. Wideman*, S. Stein* and A. Karlin. Depts. of Biochem. and Neurol., Columbia University, New York, NY 10032, and Biopoly. Res. Dept., Hoffmann-La Roche Inc., Nutley, NJ 07110

The presence of a readily reducible disulfide bond within 1 nm of the ligand binding site of nicotinic acetylcholine receptors (AChR) has been firmly established. Reduction of this disulfide bond results in profound alterations in the functional properties of these receptors and renders them susceptible to affinity labeling by cholinomimetic agents with specificity for sulfhydryl groups. Examples of such affinity labels are 4-(N-maleimido)benzyltrimethylammonium (MBTA), an antagonist, and bromoacetylcholine (BAC), an agonist. Radioactive forms of these affinity labels have been used to demonstrate that the site of specific labeling occurs exclusively on the α subunit.

The determination of the primary sequences of all four chains of Torpedo californica AChR by Noda et al. (Nature 302(1983)528) has greatly facilitated efforts to identify amino acid residues susceptible to chemical labeling. For the α subunit, Noda et al. predict that four cysteines occur in an N-terminal extracellular domain: Cys 128, 142, 192 and 193, and that the binding site disulfide bond involves some combination of these residues. Cysteines homologous to those at positions 128 and 142 occur in each of the four subunits, while those at positions 192 and 193 occur uniquely in the α subunit.

We have identified cysteine residues contributing to the binding site disulfide bond. Purified AChR from T. californica was subjected to mild reduction and was affinity-alkylated with ^{3}H-MBTA. The labeled receptor was fully reduced and carboxymethylated, and the individual subunits were separated by preparative slab gel electrophoresis. The α subunit was cleaved with cyanogen bromide, and the peptide fragments were separated by reverse phase HPLC. Sixteen fragments are predicted from the primary sequence; over 20 peaks were observed. A single peak contained 75% of the recovered radioactivity; this peak was rechromatographed and subjected to gas-phase sequencing. The first five residues obtained correspond to a unique cysteine-containing cyanogen bromide fragment. Moreover, in the sequencing of subfragments of this peptide, we have observed the release of radioactivity specifically in those sequencer cycles predicted to correspond to cysteine residues.

275.5 EVIDENCE FOR THE PRESENCE OF A SINGLE α-BUNGAROTOXIN BINDING SITE IN THE ACETYLCHOLINE RECEPTOR FROM CHICK SKELETAL MUSCLE. J. Schmidt, R. Ongjoco*, C. Adee* and B.H. Shieh*. Depts. of Biochemistry and Pharmacology, State Univ. of New York at Stony Brook, NY 11794.

We have studied the stoichiometry of interaction of α-bungarotoxin (αBuTx) and the acetylcholine receptor (AcChR) from chick skeletal muscle. In contrast to the AcChR from Torpedo californica electric tissue and the BC3H-1 mouse cell line, which contain 2 binding sites for αBuTx, the AcChR from chick skeletal muscle appears to have only one such site, as is suggested by several lines of evidence:

(a) Radioligand destruction analysis: Storage with an excess of ^{125}I-αBuTx of high specific activity inactivates receptor from denervated chick muscle and cultured chick myotubes at the rate at which the αBuTx molecule itself decomposes. This contrasts with the case of Torpedo AcChR which is inactivated at a rate consistent with double-site occupancy by ^{125}I-labeled toxin.

(b) Antigenic modulation: Antibodies to αBuTx bind to toxin-labeled AcChR on cultured embryonic chick myotubes but fail, even at the highest concentrations, to bring about acceleration of receptor turnover. This contrasts with the situation in rat myotubes where anti-αBuTx antibodies have been shown to reduce receptor half-life.

(c) Mixed derivative approach: Chick skeletal muscle AcChR, when adsorbed to agarose-αBuTx, is incapable of binding ^{125}I-αBuTx, while Torpedo AcChR retains binding activity under identical conditions.

(d) Specific activity measurements: Although chick muscle receptor resembles electroplax AcChR in sucrose density gradients and in gel permeation chromatography and therefore has a similar molecular weight, we have not been able to purify it beyond approximately 50% of the specific activity of Torpedo AcChR (4.0; 3.5; 2.9; 2.2; and 2.1 nmol per mg protein for the five best preparations vs. ca 7 nmol per mg for Torpedo).

Preliminary results suggest the presence of more than one α-subunit per chick muscle AcChR. We therefore propose that the peculiar toxin binding behavior is not caused by an unusual subunit composition but by a marked asymmetry in the binding properties of the two acetylcholine binding sites likely to be present in the receptor.

275.6 MONOCLONAL ANTIBODIES TO ELECTRIC ORGAN ACETYLCHOLINE RECEPTOR BIND TO FROG OPTIC TECTUM. Peter B. Sargent, Susan H. Pike*, Larisa Tsavaler* and Jon M. Lindstrom. Department of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305 and Receptor Biology Laboratory, The Salk Institute for Biological Studies, San Diego, CA 92138.

One approach to the study of nicotinic acetylcholine receptors (nAChRs) in the central nervous system is to utilize cross-reacting monoclonal antibodies (mAbs) prepared against nAChRs from electric organ. In the present study 126 mAbs made against nAChRs from electric organs of Torpedo californica or Electrophorus electricus were tested for their ability to bind to the optic tectum of frog (Rana pipiens). Antibody binding was assayed in cryostat and in vibratome sections of tissue using the avidin-biotin-peroxidase complex technique (ABC technique).

Many of the mAbs which bind to nAChRs in frog skeletal muscle also cross-react in the tectum. The staining pattern produced by cross-reacting antibodies is restricted to a pair of prominent bands that occupy much of the optic neuropil. The staining appears to be similar, but not identical, to the termination sites of retinal ganglion cells as determined in other laboratories.

mAbs specific for each of the four subunits of electric organ nAChR (α, β, γ, δ) produced similar staining patterns in the tectum. Among the cross-reacting mAbs are several antibodies which are specific for the "main immunogenic region" of the α subunit. Cross-reacting mAbs include antibodies which recognize extracellular domains of the nAChR as well as those which recognize intracellular domains.

These results suggest that the optic tectum contains molecules structurally similar to nAChRs. The distribution of these molecules is consistent with their involvement in retinotectal synaptic transmission. Further support for such a role awaits the fine structural localization of antibody binding and a careful comparison between the pattern of antibody binding and of retinal ganglion cell termination.

Supported by the NSF, March of Dimes, and American Heart Association (to P.B.S.) and by the NIH, McKnight Foundation, and Myasthenia Gravis Foundation (to J.M.L.).

275.7 CHARACTERIZATION OF A COMPONENT IN CHICK CILIARY GANGLIA THAT CROSSREACTS WITH MONOCLONAL ANTIBODIES TO ACETYLCHOLINE RECEPTOR FROM MUSCLE AND ELECTRIC ORGAN. M.A. Smith, J. Stollberg, D.K. Berg, and J.M. Lindstrom. Dept. of Biol., UCSD, La Jolla CA. 92093; and The Salk Institute, S.D., CA. 92138.

Chick ciliary ganglion neurons have nicotinic acetylcholine (ACh) receptors that mediate the only known form of chemical transmission through the ganglion. Ultrastructural studies have demonstrated that monoclonal antibodies (mAbs) to a determinant in the main immunogenic region (MIR) of muscle and electric organ ACh receptors also crossreact with a component on ciliary ganglion neurons located predominantly in the postsynaptic membrane. We have used a radiolabeled mAb to examine the neuronal component further.

The amount of crossreacting antigen in detergent extracts of embryonic chick tissues was measured using an ^{125}I-labeled anti-MIR mAb (mAb 35), and ion exchange chromatography to isolate antigen-antibody complex. Ciliary ganglia from 17-18 day embryos contain about 4 fmoles of high affinity (K_D = 1 nM) mAb 35 binding sites per ganglion. The binding is specific in that it can be blocked by other anti-MIR mAbs of the same specificity but not by non-immune serum. The amount of MIR-like component increases 4-fold between embryonic days 10 and 18. Previous ultrastructural studies revealed a difference in the distribution of mAb 35 sites and alpha-bungarotoxin sites on the neurons. The present results indicate that the neurons have about 5 times more toxin sites than mAb 35 sites. Moreover, depletion of the MIR-like component from detergent extracts by mAb 35 together with secondary antibody precipitates less than 4% of the toxin sites.

Sympathetic ganglia, which also have ganglionic ACh receptors, have similar levels of MIR-like component per mg protein. Little or no mAb 35 binding can be detected in detergent extracts of retina, spinal cord, dorsal root ganglia, ventricular heart muscle, or liver.

Concanavalin A has been reported by A. Messing to block ACh receptor function on ciliary ganglion neurons, suggesting that the lectin binds to the receptor. We find that concanavalin A coupled to Sepharose beads quantitatively depletes mAb 35 binding sites from ganglion extracts; alpha-methyl-D-mannoside elutes 80% of the absorbed sites.

The MIR-like component in chick ciliary ganglia displays several properties expected for a ganglionic ACh receptor. (Supported by NS 12601, The Muscular Dystrophy Assoc., & The American Heart Assoc.)

275.8 A HIGH-MOLECULAR WEIGHT BUNGAROTOXIN-BINDING COMPONENT IN FISH AND AVIAN BRAIN DETECTED AND CHARACTERIZED USING PROTEIN BLOTS. Edward Hawrot, Paul T. Wilson*, Jonathan M. Gershoni*, Andrea L. Boissevain*, and Thomas L. Lentz. Depts. of Pharmacology and Cell Biology, Yale University School of Medicine, New Haven, CT.

We demonstrated previously the binding of ^{125}I-labeled bungarotoxin (BGTX) to the dissociated α-subunit of Torpedo acetylcholine receptor (AChR) using a modified "protein-blot" analysis. The denatured subunits of the Torpedo AChR are resolved by polyacrylamide gel electrophoresis and electrophoretically transferred to positively charged nylon filters which can then be incubated directly with labeled BGTX. This approach also has been used to detect BGTX binding to proteolytic fragments of the α-subunit (Wilson et al., PNAS, in press).

We have now used this same approach to investigate the physical characteristics of BGTX-binding components found in central nervous system tissue. We have examined avian and fish brain material since there is some electrophysiological evidence that the BGTX-binding component in these species represents a nicotinic, neuronal AChR. Triton extracts of avian or fish brain were enriched for the binding component by affinity chromatography on BGTX-Sepharose. The bound material was eluted with lithium dodecyl sulfate sample buffer and electrophoretically resolved and analyzed by protein blotting with labeled BGTX. We obtained BGTX binding to a high-molecular weight brain component (approximately 200,000 daltons) under conditions where the Torpedo AChR is completely dissociated. The binding of BGTX to the protein blots of brain material could be blocked by nicotine or d-tubocurarine but was unaffected by atropine. Boiling the brain extracts destroyed the BGTX binding activity whereas a similar treatment had little or no affect on Torpedo AChR binding activity.

These results indicate that a high-molecular weight BGTX-binding component from brain exhibits nicotinic pharmacology when assayed on protein blots. Furthermore, there appear to be significant structural differences between muscle and neuronal BGTX-binding sites treated under identical conditions. Supported by NIH GM 32629, the American Parkinson Foundation, the PMA Foundation, and NSF 82-03825.

275.9 α-BUNGAROTOXIN, BROMOACETYLCHOLINE AND ANTI-NICOTINIC CHOLINERGIC RECEPTOR ANTIBODY BINDING SITES ON THE PC12 PHEOCHROMOCYTOMA LINE. R.J. Lukas. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Putative nicotinic acetylcholine receptor (nAcChoR) sites on the rat pheochromocytoma cell line PC12 have been detected on the basis of their interactions with three presumably nAcChoR-specific probes. High affinity ($K_D \cong$ 1nM) sites for α-bungarotoxin (Bgt) have been detected at a site density of 10 fmol per 10^6 cells. Extensive ligand competition experiments conducted on cells in suspension indicates that d-tubocurarine, nicotine and acetylcholine are the most potent inhibitors of high affinity Bgt binding. The nAcChoR-specific affinity alkylating reagent, bromoacetylcholine (BAC), reacts irreversibly with dithiothreitol-reduced PC12 cells in suspension, and blocks high affinity Bgt binding. BAC interaction with toxin binding sites is blocked if reduced cells are first reacted with non-specific alkylating agents, such as N-ethylmaleimide. The sequence of dithiothreitol-mediated reduction and non-specific, N-ethylmaleimide-mediated alkylation of toxin binding sites that interact with BAC is blocked if PC12 cells are preincubated with nicotinic agonists, but not antagonists. In addition, affinity alkylation of reduced toxin binding sites is also prevented when cells are incubated in the presence of nicotinic agonists. At the highest concentrations tested, monoclonal antibodies raised against nAcChoR from Torpedo electroplax and each of two polyclonal antisera raised against Electrophorus nAcChoR immunoprecipitate less than 5% of detergent-solubilized PC12 cell Bgt binding sites, while an anti-toxin antiserum quantitatively precipitates toxin:PC12 cell toxin binding site complexes. However, both polyclonal anti-nAcChoR antisera inhibit high affinity toxin binding to sites on PC12 cells in suspension. Taken together, these results indicate that PC12 cell high affinity Bgt binding sites share properties with similar sites on mature rat brain crude mitochondrial fractions. These sites are recognized by the nAcChoR agent, BAC, and by anti-Electrophorus nAcChoR antisera. Since these antisera have been shown to exhibit physiologically-relevant antagonistic potency against functional cholinoceptive sites on the PC12 clone, these data support identification of brain and PC12 cell high affinity Bgt binding sites as a subclass of a potentially heterogeneous population of neuronal-like nAcChoR.

275.10 ENDOGENOUS INHIBITOR OF LIGAND BINDING TO THE MUSCARINIC ACETYLCHOLINE RECEPTOR. R. Diaz-Arrastia, T. Ashizawa*, and S. H. Appel, Dept. of Biochemistry and Neurology and Program in Neurosciences, Baylor College of Medicine, Houston, TX 77030.

A novel inhibitor of ligand binding to the brain muscarinic acetylcholine receptor (MAchR) was identified. [3H]Quinuclinidinyl benzilate ([3H]QNB) binding to rat brain synaptosomes was measured using a filtration assay. The inhibitor was present in several calf tissues and was found in highest specific activity in the thymus. The loss of binding activity was slow, requiring a 30-40 minute preincubation of the synaptosomes with the inhibitor before the maximal effect was seen. Early on, the inhibition is due mainly to a decreased affinity of the receptor for [3H]QNB, but after an hour preincubation with the inhibitor the effect is mainly due to a decrease in the Bmax. Removal of the inhibitor by washing the synaptosomes reverses the loss of binding activity. Zn2+ is required at low concentrations for the effect; inhibition is blocked by EDTA and restored by adding Zn2+. Intact synaptosomes are not required. MAchR in synaptic membranes and in membranes free of most peripheral membrane proteins was still sensitive to inhibition.

Preliminary characterization of the inhibitory molecule showed that it is of low molecular weight, moderately heat stable and acidic. Approximately a 20,000 fold purification has been achieved from the crude high-speed supernatant by boiling, acid extraction, gel filtration and anion exchange chromatography. This research supported by NIH 470-G11569 and grants from the John A. Hartford Foundation and the Helen C. & Robert J. Kleberg Foundation.

275.11 A MYASTHENIA GRAVIS SERUM BLOCKS AChR FUNCTION IN C2 MYOTUBES. Yong Gu*, Shaul Hestrin*, Villu Maricq* and Zach W. Hall, Div. Neurobiol., Dept. Physiol., Univ. Calif., San Francisco, CA 94143

The sera of patients with myasthenia gravis contain antibodies against the acetylcholine receptor (AChR), but relatively few sera have been shown to directly affect its physiological function. We have described previously a myasthenic serum of unusual specificity (Hall et al, 1983). In rat and mouse muscle, this serum partially blocks the binding of α-bungarotoxin to AChRs from the extrajunctional membrane of embryonic or denervated fibers, but does not affect the AChR at adult neuromuscular junctions. Using myotubes from the cell line C2, we showed that the block of toxin-binding is partial because the antibody specifically recognizes only one of the two toxin-binding sites on each AChR molecule.

Because two agonist molecules must bind to each AChR to fully activate the channel, we have tested the effect of this antibody on the function of the AChR in C2 myotubes. Treatment of the cells with antibody reduced the carbamylcholine-induced ^{22}Na influx. Although block of toxin-binding by antibody was never more than 50%, up to 80% inhibition of the ^{22}Na response could be obtained. Over a range of serum concentrations up to 3 mg/ml, the data were consistent with a model in which binding of antibody to a single toxin-binding site blocks AChR function. The inhibition of ^{22}Na influx by antibody was not relieved by higher concentrations of carbamylcholine.

We further investigated the effect of the serum using patch clamp techniques. Comparison of patches taken from normal and antibody-treated cells showed no change in the single-channel conductance nor in the mean channel open time. The frequency of events seen in patches taken from antibody treated cells, however, was generally reduced. These data, taken together with the ^{22}Na data, suggest that the antibodies in the serum completely block the function of individual channels. The residual ^{22}Na influx seen with antibody could result from failure to achieve saturation or to a population of resistant channels.
Supported by NIH and MDA.

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275.12 SEVEN GENES AFFECT THE BINDING PROPERTIES OF THE NEMATODE LEVAMISOLE RECEPTOR. J.A. Lewis, J.S. Elmer*, J. Skimming*, S. McLafferty*, J. Fleming* and T. McGee*. Dept. of Biological Sciences, University of Missouri, Columbia, MO 65211

Receptor mutants of the nematode Caenorhabditis elegans can easily be isolated by selection for resistance to the neurotoxic compound levamisole. Levamisole is a nicotine-like drug that causes muscle contraction of the wild type worm. We show that the most levamisole-resistant mutants probably escape poisoning by their inability to produce a normal levamisole receptor and thereby define a set of 7 genes necessary for proper expression of this receptor. Levamisole receptor activity was assayed by the binding of tritiated meta-aminolevamisole (^3H MAL). The wild type ^3H MAL binding activity is composed of a saturable high affinity component and a nonsaturable background activity. The cholinergic blocking agent mecamylamine, which pharmacologically blocks the muscle-contracting effects of levamisole, acts as an apparent allosteric activator of the saturable wild type ^3H MAL binding activity. Mutants of 3 genes (unc-29, unc-50, and unc-74) appear devoid of high affinity activity and the residual nonsaturating component is not activated by mecamylamine. Mutants of the 4 remaining resistance loci (unc-38, unc-63, lev-1, and lev-7) all possess significant amounts of saturable ^3H MAL binding activity but binding in these mutants is not usually potentiated by mecamylamine. The receptor present in mutants of 3 of these genes (unc-38, unc-63, and lev-7) has a much higher affinity for ^3H MAL than does the wild type in the absence of mecamylamine. These results suggest the possibility that the ^3H MAL binding subunit of the receptor originates in a nascent high affinity state and is converted to the lower affinity state seen in the wild type upon formation of a complete, intact receptor. Mutants of the lev-1 locus differ in that the receptor in these mutants is usually found in the lower affinity state, like the wild type, but is not activated by mecamylamine.

We have been able to purify the levamisole receptor 30,000 to 60,000 times over its specific activity in crude, total homogenates of the wild type worm, to a purity of a few percent. We may thus soon be able to better define receptor defects in these mutants through the use of monoclonal antibodies.

ACTION POTENTIALS AND ION CHANNELS VI

276.1 LOSS OF A-CURRENTS AND PRESERVATION OF Ca CURRENTS DURING HORMONE-INDUCED MEMBRANE AREA DECREASE IN STARFISH OOCYTES. W.J. Moody* and M.M. Bosma*(SPON:M.S. Letinsky). Dept. of Zoology, Univ. of Wash., Seattle, WA 98195, and Dept. of Physiology, UCLA, Los Angeles, CA 90024.

Immature oocytes of the starfish, L. hexactis, are electrically excitable and under voltage clamp conditions display three principal ionic currents: an inward Ca current, a fast transient K current (A-current), and an inwardly rectifying K current. During meiotic maturation of the oocyte, which can be carried out in vitro in about one hour using the endogenous starfish hormone 1-methyladenine, the amplitude of the A-current decreases substantially while the Ca current either becomes larger or does not change (Moody and Lansman, PNAS, 80:3096, 1983). We are interested in the mechanism by which the hormone has such substantial and different effects on these two conductances.

Total membrane capacitance measured under voltage clamp decreases by more than 50% during maturation and electron microscopic examination of the surface membrane before and after hormone treatment indicates a dramatic loss of microvilli and flattening of the membrane which could account for the decline in total capacitance. The slope conductance of the A-current was measured at various times after the beginning of hormone treatment and compared with capacitance measurements in the same cell. After one hour of exposure to hormone, cell capacitance had declined to 48±6% (n=7) of its initial value, while A-current conductance had decreased to 50±8% of its initial value. Both changes were gradual over the hour and the time courses were virtually identical. From these data we conclude that 1-methyladenine triggers a loss of surface membrane including a proportional loss of A-current channels. Because both total capacitance and A-currents partially recover during prolonged hormone treatment, we suspect that the membrane is taken into the cell in a form which permits its reinsertion later as part of the cell surface. We do not know how the Ca currents are spared while approximately 50% of the surface membrane is removed, but it is possible that the Ca channels are clustered in a region of the cell surface which is protected from the actions of the hormone.

276.2 INCREASE OF OUTWARD CURRENTS DURING DIFFERENTIATION OF AMPHIBIAN NEURONS IN VITRO. M.E. Barish (SPON: S. Hagiwara), Department of Physiology, UCLA School of Medicine, Los Angeles, CA 90024.

The whole cell variant of the patch clamp technique has been used to study the membrane currents of embryonic neurons as they differentiate in vitro. These experiments are aimed towards describing the pattern of development of neuronal cell membranes.

Cultures were made from dissociated neuronal precursor cells isolated from Ambystoma mexicanum embryos at (Harrison) stages 14 to 16. At these stages the primordial nervous system consists of the neural plate and surrounding neural folds. Only cells from the rostral half of the neural plate were placed into culture. In a serum-free medium with added growth supplements cultures could be maintained for at least one week. Whole cell current was recorded with patch electrodes with openings of about 1.5 μm. To maintain space clamp, neurites were severed from the cell soma with a microelectrode.

At day 1 in culture (plating was day 0), no cells with neurites were observed. Under voltage clamp, some cells showed time-dependent outward current, but no inward currents could be recorded. Although there was no independent criterion other than their original source by which to identify these cells as neurons, this result suggests that one of the first electrical signs of neuronal differentiation may be the appearance of outward rectification. Cells with neurites appeared between day 2 and day 3. Neurons were mono-, bi- or tri-polar, and had spherical or elliptical somas 15 to 25 μm in diameter. Over the first four days, the average steady state outward current in recordings of total membrane current progressively increased. Average values (mean ±s.d., in nA recorded at +40 mV) were -- day 1: 0.18 ±0.08; day 2: 0.73 ±0.58; day 3: 1.09 ±0.68; day 4: 2.36 ±2.18. The considerable scatter in the data probably reflects heterogeneity in the types of neurons in the cultures and in their state of differentiation, as well as variability in their size. Nevertheless, a steady increase in outward current amplitude seems to occur during differentiation, and this increase may exert a large influence on the waveform of the soma action potential. Future experiments are aimed towards quantifying the changes in conductance and kinetics of individual K currents, and assaying their importance in relation to parallel changes occuring in inward currents in modulating the duration and ionic sensitivity of the action potential.

276.3 IONIC CURRENTS AND CHANNELS IN GROWTH CONES OF IDENTIFIED APLYSIA NEURONS. F. Belardetti*, S. Schacher, and S. A. Siegelbaum. Center for Neurobiology & Behavior, Dept. of Pharmacology, Columbia Univ., College of P & S, and N. Y. State Psychiat. Instit., N.Y., N.Y. 10032.

Growth cones are a specialized area of neurons, essential for synaptogenesis, whose electrophysiological properties have been difficult to study with conventional intracellular methods. Here we report experiments using the patch clamp technique to record directly electrical signals from growth cones of right upper quadrant (RUQ) neurons of Aplysia abdominal ganglia grown in culture (Flaster et al., Soc. Neurosci. Abstr., 1984).

A high resistance gigahom seal was obtained with a patch pipette on growth cones of RUQ neurons 3-7 days in culture. The patch of membrane under the pipette was then ruptured to allow a voltage clamp of the whole growth cone. Roughly one-third of all growth cones display a large (460 ± 297 pA; mean ± S.D., N=10) rapidly activating and inactivating inward current. This current has the expected properties of the rapid sodium current. Following a depolarizing command step, the current reaches its peak inward value within 1-2 msec and then inactivates along an exponential time course with time constant of 1-5 msec. The current is half inactivated at a holding potential of -30 mV, reaches half maximal activation in response to depolarizations to +5 mV, and is completely blocked by 50 um TTX. The inward current is followed by the turning on of both a transient (I_A) and delayed outward potassium current. These growth cones exhibit large overshooting action potentials under current clamp. The remaining two-thirds of the growth cones display only outward currents under voltage clamp and tend to be either inexcitable or display only small action potentials under current clamp.

To confirm that the ionic currents are indeed arising from growth cone membrane, single channel currents were recorded from growth cones in cell-attached patches. Several species of channels have been observed: 1) A rapidly activating and inactivating inward current channel whose properties conform to the whole growth cone macroscopic inward current. The channels have a unit conductance of 4-8 pS and display a net current amplitude of around -.5 pA at around 0 mV. Such channels are seen in approximately 15% of all patches. 2) A more slowly activating outward current channel was observed in response to large positive voltage steps (+100 mV above rest). This channel has a conductance of around 40 pS and resembles the calcium-activated potassium channel observed in intact Aplysia neurons. 3) A smaller outward current channel was also observed in response to strong depolarizations with a unit conductance of 10 pS.

276.4 **VOLTAGE GATED ION CHANNELS IN NEURONAL GROWTH CONES.** **Allan S. Greenberg and Ilan Spector, Depts. Neurobiology and Anatomical Sciences, SUNY, Stony Brook, 11794**

Single channel currents were recorded with the cell attached patch clamp technique from growth cones of DMSO-differentiated N1E-115 neuroblastoma cells. When the bath and the pipette contained normal saline most membrane patches did not display any spontaneous channel openings at rest or at more negative potentials. From a holding potential of -50 mV, depolarizing steps to 0 mV (relative to rest) and to more positive levels disclosed three major classes of voltage-gated channel events that could be distinguished by their amplitudes and activation voltage level. Inward currents always appeared with steps to 5±5mv (relative to rest). Patches depolarized to more positive levels also exhibited a population of outward currents. At even greater depolarizations, a class of larger outward currents was observed in 40% of the patches with about 3 times the amplitude of the first type. The frequency of openings of this large channel increased after excising the patch in Ca-containing solutions. The inward currents are considered to result from Na channels because they were eliminated when TTX was added to the pipette solution, had short open times, and inactivated very rapidly. When TEA (10mM) was added to the pipette, voltage-gated outward currents were not observed indicating that these currents result from K channels. Under these conditions the I-V curve of the inward currents showed a linear portion with a slope of about 14 pS and an extrapolated reversal potential of 123 mV (+rest). Both classes of outward currents inactivated during maintained depolarizations, and responded to increasing depolarizing steps with increased frequency of channel opening, prolonged open times and abbreviated latency-to-first-opening. Their conductance and extrapolated reversal potential (relative to rest), based on the linear portion of the I-V curve were 41 pS; -27 mV for the small events, and 136 pS; -13 mV for the large ones. When the patch electrode contained TTX, TEA and isotonic $CaCl_2$ or $BaCl_2$, small inward currents (<1 pA) were observed which may represent a population of Ca channels. This study presents the first direct evidence that a variety of voltage-gated channels exist in the growth cone membrane. The abundance of Na and K channels in this specialized region further indicates that these conductances may dominate its electrical behavior.

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276.5 DEVELOPMENT OF THE VOLTAGE DEPENDENT SODIUM CHANNEL IN CULTURED RAT DORSAL ROOT GANGLION CELLS- IMMUNOFLUORESCENCE STUDIES. H.Meiri,G.Omri*,I.Zeitoun*, G.M.Gershoni & N.Savion*. The Sackler Sch. of Med. Tel Aviv Uni. Ramat Aviv,69978 and The Weizmann Ins. for Sci. Rehovot, 76100,israel.

The development of sodium dependent action potential was studied in cultured dorsal root ganglion(DRG) cells derived from new-born rats using immunofluorescence techniques with the following sodium channel specific monoclonal antibodies(mAb's):SC-72-14 blocks the channel primarily by shifting the voltage dependent inactivation system towards hyperpolarization as revealed by voltage clamping the node of Ranvier of rat peripheral nerve. These mAb's also stain the node and the staining is abolished by veratridine.SC-72-38 induces repetitive firing by shifting the voltage dependent activation system towards hyperpolarization.These mAb's not only compete with Tityus-γ-toxin for the binding to the sodium channel but also mimic its electrophysiological effects. Thus, these mAb's recognize sites associated with the channel activity in the mature neuron.Their corresponding antigen appears to be a 225-250 kd polypeptide as determined by immunoblotting. These mAb's were used to follow channel appearance in DRG cells cultured in defined conditions that regulate their differentiation.

DRG cells send neurites when cultured in serum free medium(SFM) on poly-L-Lysine(PLL)coated dishes. When they were cultured on extracellular matrix(ECM),produced by bovine corneal endothelial cells,outgrowth was further stimulated.The nerve growth factor(NGF), enhanced both neuronal outgrowth and cell survival. Addition of 1% fetal calf serum(FCS) to all growth conditions increased cell survival.The high density lipoprotein(HDL) caused nerve cell aggregation but outgrowth and survival were not improved.

Immunofluorescence detection of the channels in the above growth conditions revealed the following picture: When the cells were cultured in SFM on either PLL or ECM coated dishes channels were found on cell bodies only.NGF and FCS induced channel appearance in the main neuritic branches..HDL increases channel density in all growth conditions. Moreover, it induced channel appearance in the neurites of DRG cells grown in SFM on PLL or ECM coated dishes.

276.6 VOLTAGE-DEPENDENT CONDUCTANCES IN CULTURED SYMPATHETIC NEURONES DURING DEVELOPMENT. J. M. Nerbonne, A. M. Gurney and H. Rayburn*. Biology Division, Caltech, Pasadena, CA 91125.

The whole-cell patch clamp recording technique was employed to study the development of electrically excitable membrane properties in superior cervical ganglion neurones in culture. Cells, isolated from newborn rats by enzymatic digestion, were grown in monolayer cultures in MEM supplemented with 10% horse serum and NGF. Within 2 hrs after plating, cells adhered to the substrate and whole-cell recordings could be obtained. Under control conditions (140 mM Na^+ and 10 mM Ca^{++} in the bath and 140 mM K^+ in the pipette), depolarizing voltage steps from holding potentials of -30 to -50 mV revealed fast inward TTX-sensitive Na^+ (I_{Na}) and delayed outward K^+ (I_K) currents. In the absence of I_{Na} (1 μM TTX) and at hyperpolarized holding potentials (-70 to -90 mV), depolarization activated a transient outward K^+ current (I_A) in addition to I_K. Replacement of K^+ with Cs^+ (140 mM) in the pipettes blocked outward K^+ currents and revealed the presence of a slow inward current blocked by Co^{++} and carried by Ca^{++} (I_{Ca}). All of these currents are present in cells studied at times 4 hrs to 6 wks after plating and, in addition, the kinetics, ion- and voltage-dependences of the currents are similar at all stages. The results, taken together, suggest that these voltage-dependent conductances develop in sympathetic neurones before birth.

Qualitatively, the ionic currents measured in whole-cell recordings here are similar to those seen using microelectrode voltage clamp techniques (J. E. Freschi, J. Neurophysiol., 50, 1460 (1983)). However, we find that the steady-state current-voltage relation for I_{Ca} peaks at +30 mV and that I_{Ca} displays little or no inactivation (<10%) during 500 ms voltage steps to -10 to +50 mV (E_H = -50 mV). We assume that the differences between our results and those reported previously arise from the ease of separation of I_{Ca} from contaminating outward K^+ currents using the whole-cell technique.

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276.7 DEVELOPMENT OF MEMBRANE EXCITABILITY IN RAT DIENCEPHALIC NEURONS IN SERUM-FREE CULTURE. Z. Ahmed, J.A. Connor, D.W. Tank and R.E. Fellows. Dept. of Physiology, Univ. of Iowa, Iowa City, IA 52242 and AT&T Bell Laboratories, Murray Hill, NJ 07090.

Studies on developing neurons from different animal sources have shown a wide variation in the order in which ionic channels appear in the membrane. Using the whole cell gigaseal voltage clamp technique we have investigated the expression of voltage dependent ionic currents in dissociated primary cultures of fetal rat (E17) dienceph-alic neurons grown in a serum-free defined medium (Ahmed et.al. J. Neurosci. 3:2448). At this date we have been able to characterize at least two populations of neurons with different developmental properties. One class of cells first expressed a transient inward current after 10-24 hr in culture. These transient, all-or-none, inward currents arose at the cell neurites where the membrane voltage was not under space-clamp. The all-or-none inward current could be reversibly blocked by either 1uM tetrodo-toxin (TTX) or 1mM cobalt. No appreciable outward current was present in the cells at this stage. With further maturation the magnitude of the all-or-none current was increased. After about 6 days in culture these cells developed a large, inward current whose activation was graded with voltage. At this stage a transient outward current was also present. In external cobalt the inward current showed a small reduction in amplitude but an increase in duration. The transient outward current was abolished. In TTX more than 85% of the inward current was blocked but the outward transient remained. The outward transient appeared to consist of both an A-current and a calcium activated transient. The second population of neurons expressed a voltage dependent outward current in the soma after 20 hr in culture. After 3 days these cells expressed a voltage-dependent TTX-sensitive, cobalt insensitive inward current in the soma which showed the typical I-V relationship observed in other vertebrate neurons. Currents increased in magnitude with additional time in culture. Supported by NIH HL24402, F32NS06977 and AT&T Bell Laboratories.

276.8 Ca^{2+} ACTIVATED K^+ CHANNELS IN GLIAL CELLS. Fred N. Quandt and Brian A. MacVicar. Dept. Med. Physiology, University of Calgary, Faculty of Medicine, Calgary, Alberta, Canada T2N 4N1.

Glial cells from newborn rat, grown in tissue culture have been found to exhibit Ca^{2+} spikes when exposed to tetraethylammonium (TEA; MacVicar, this meeting). TEA could block one of several species of K^+ channels to produce this effect. Single channel analysis was applied in order to identify the types of K^+ channels present in these cells. Glial cells were prepared in primary cultures using the methods of Booher and Sensenbrenner (1972) and Kimmelberg et al. (1976). Recordings were obtained from cultures, from 2 to 8 weeks old, following exposure to 1mM dibutyryl-cAMP. Glial cells were identified on the basis of morphology; and positive immunohistochemical staining for glial fibrillary acidic protein, a specific glial marker. Single channel currents were recorded from intact or inside-out plasma membranes of glial cells using the gigaohm seal, patch clamp technique. Upon depolarization, outward current steps could be observed consistently from inside-out membranes in the presence of internal solution containing 10 to 100uM Ca^{2+}. These outward currents were suppressed following the removal of Ca^{2+} but resumed when Ca^{2+} was reapplied. Channels gating in the presence of Ca^{2+} appeared to be primarily permeable to K^+, since substitution of internal K^+ with either Cs^+ or tetra-methylammonium eliminated the currents reversibly. Gating of the channel appeared to be voltage-dependent in the presence of Ca^{2+}, since the frequency of opening increased with depolarization. The currents exhibited bursting behavior with a typical burst duration of 45 msec (-20 to 0mV, 12°C, 10 uM Ca^{2+}). Current amplitude increased as the membrane was depolarized away from E_K. The slope conductance between -20 and 0mV was 52 pS. The channels were blocked by internal TEA (20mM) but not 4-aminopyridine (200uM). The currents can be best classified as arising from a Ca^{2+}-dependent K^+ channel in the glial cell membrane. These results, combined with the evidence for a voltage depenent Ca^{2+} channel, suggest a dynamic role for glial cells in controlling excitability in CNS structures. Influx of Ca^{2+} would change membrane permeability to K^+ and possibly lead to K^+ efflux from the glial cells. Supported by the Medical Research Council of Canada and the Alberta Heritage Fund for Medical Research.

276.9 PATCH CLAMP STUDIES ON MURINE SPINAL CORD NEURONS IN CELL CULTURE.
D.A. Mathers* (Spon: J.A. Pearson), Dept. of Physiology, University of British Columbia, Vancouver, British Columbia, V6T 1W5.

The techniques of primary cell culture and extra-cellular patch clamp recording were used to study sponta-neous and agonist induced membrane channels in murine spinal cord neurons. Cells were obtained from adult rat dorsal root ganglia (DRGs) and from embryonic mouse spinal cord tissue. It was found that membrane patch-electrode seals of $>10^9\ \Omega$ could be readily obtained using both cell types. Most patches studied exhibited spontaneous single channel currents even in the absence of the puta-tive neurotransmitters γ-aminobutyric acid (GABA) and glycine. In the case of adult rat DRG neurons, spontaneous inwardly directed single channel currents were seen in patches voltage clamped at the resting potential (-40 to -50 mV). In 6 patches studied in detail, these events showed a single channel conductance of 23 $\pm$ 5.6 pS (mean $\pm$ S.D.) and an apparent reversal potential close to 0 mV. Histograms of both open and closed time intervals were not well fit by single exponentials, indicating deviation from a simple two state model of channel function. These events resemble currents reported in cultured heart cells and attributed to cation specific channels with little selectivity for Na^+ over K^+ ions.

In cultured mouse spinal cord cells, large, spontaneous outward currents were frequently seen in patches voltage clamped at 0mV. These events displayed a mean single channel conductance of 180 pS and a reversal potential predicted by the Nernst potential for K^+ ions. They resemble Ca^{2+} dependent, K^+ selective channels seen in many other neurons. Application of 10 μM GABA or glycine to embryonic mouse spinal cord cells invariably resulted in a flow of current which could be detected using the whole cell clamp technique. Experiments using outside-out patches suggested that both amino acids alter membrane currents by opening previously inactive channels in the neuronal membrane.

276.10 INTRACELLULAR AND SINGLE CHANNEL ANALYSIS OF VOLTAGE-SENSITIVE IONIC MECHANISMS IN THE SOMAL AND DENDRITIC MEMBRANES OF CULTURED CEREBELLAR PURKINJE NEURONS. D.L. Gruol. Division of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, CA 92037 USA.

A well characterized CNS neuronal type, the cerebellar Purkinje neuron (PN), was selected as a model to examine the ionic mechanisms underlying neuronal excitability in the vertebrate CNS and to reveal the topographic organization of these mechanisms across the neuronal surface. An in vitro model system, cultured rat PNs, was used. Extracellular recordings from the cultured PNs revealed patterns of spontaneous activity similar to that observed in vivo. Intracellular recordings from the soma and dendritic regions and pharmacological agents or ions known to block specific ion channels were used to identify ionic mechanisms that contribute to the patterns of activity. Two common patterns were identified as arising from intrinsically generated, voltage-sensitive mechanisms. Synaptically evoked activity was also observed. Na+ and Ca++ channel blockers (0.5 μM TTX; 10 mM Mg++) totally abolished the spontaneous activity, including that attributed to intrinsic mechanisms. K+ channel blockers (TEA, 4-AP) altered the pattern of activity. These channel blockers also altered the voltage responses evoked by intracellular current injection. Data from this series of experiments suggest that a variety of ionic mechanisms mediate the electrical activity of PNs including: 1) a fast Na+ spike, 2) a Ca++ spike, 3) a slow Ca++ conductance, 4) a Ca++ activated K+ conductance, and 5) the delayed rectifier. Single channel recordings were used to characterize these ionic mechanisms and their regional distribution across the neuronal surface. Recordings from the somal region of PNs (cell-attached or outside-out configuration) revealed several types of membrane channels, including at least three types of voltage-sensitive K+ channels, identified by their single channel conductance, voltage sensitivity and sensitivity to TEA. Voltage-sensitive K+ channels, including a Ca++ activated K+ channel, were also observed in the dendritic region of the PNs. In both regions, K+ channel activity was associated with the falling phase of spontaneous action potentials. These data suggest that Ca++ and K+ mediated electrical events occur in both the somal and dendritic region of PNs and that K+ channels function in the repolarizing phase of action potentials, which appear to be generated in both regions. (Supported by NIAAA 06420).

276.11 PATCH CLAMP RECORDING OF VOLTAGE-DEPENDENT POTASSIUM CHANNELS IN CULTURED HIPPOCAMPAL NEURONS. Michael A. Rogawski. Laboratory of Neurophysiology, NINCDS, National Institutes of Health, Bethesda, MD 20205.

Single voltage-activated K^+ channels were first recorded in squid axon by Conti and Neher [Nature 285: 140 (1980)] using an early version of the patch clamp. Recent improvements afforded by the gigaseal technique allow the properties of K^+ channels to be studied with higher resolution and it is now possible to examine their kinetic behavior after an instantaneous change in potential.

Gigaseal patch recordings were made at room temperature from the somatic membranes of cultured hippocampal neurons from 19-day old rat embryos. Cells were bathed in tetrodotoxin (1-2 μM) and $CdCl_2$ (0.2 mM) to block Na^+ and Ca^{2+} entry, respectively. In the cell-attached configuration, depolarization of the membrane patch by 15 to 60 mV resulted in the appearance of outward unitary currents. The frequency of channel opening increased markedly with the magnitude of the depolarization. Channel amplitudes were normally distributed and varied linearly with potential; there was no evidence for subconductance states. The single channel slope conductance was 20 ± 2 pS and the extrapolated reversal potential was 21 ± 5 mV below rest, i.e., near the inversion potential of macroscopic K^+ currents in these cells. Experiments with excised patches confirm that the channels pass predominantly K^+ ions. Channel opening occurred in bursts with occasional brief flickerings to the closed state. Burst lifetimes were exponentially distributed with a time constant of 10 ± 5 msec for times < 30 msec. There were a disproportionately large number of bursts with long lifetimes, suggesting a second component to the lifetime distribution.

During identical repetitive voltage steps (0.3 to 0.5 Hz), the probability of channel opening varied in a non-random fashion with time so that there were a greater than expected number of records with no openings (χ^2 comparison with Poisson distribution) and a non-random clustering of these blank records (runs test). This suggests that the channels can enter a prolonged inactivated state.

Unitary currents occurred in Ca^{2+}-free/EGTA-containing solution, but not in tetraethylammonium (20 mM). Following a voltage command of 20 to 30 mV, the probability of channel opening increased gradually in an exponential fashion with a time constant of 100 msec. This is similar to the activation time constant of the macroscopic delayed rectifier K^+ current in these cells. Therefore the channels have kinetic and pharmacological properties expected of the unitary events underlying the delayed rectifier conductance.

276.12 THE SODIUM CURRENTS OF MOUSE SPINAL CORD NEURONS EARLY IN DEVELOPMENT. A.B. MacDermott and G.L. Westbrook, Lab.Neurophysiol.(NINCDS) & Dev. Neurobiol.(NICHD),NIH,Bethesda,Md.

During development in culture, mouse spinal cord neurons show marked increases in action potential(AP) rise rates and ^{3}H-saxitoxin binding (Westbrook,et al.,1983), and acquire repetitive firing. To examine whether these events reflect changes in number, distribution, or kinetics of sodium(Na^+) channels, we have used patch electrodes to voltage clamp the sodium currents underlying APs after 1-5 days in culture.

Spinal cord neurons were dissociated from 13 day embryonic mice. On day 1 in culture, neurons were small(~5 um dia.) with a few short neurites. Na^+-dependent APs could be evoked with intracellular current pulses in normal bathing medium. To record Na^+ currents from a region that was adequately space clamped, choline was substituted for Na^+ in the bath, and brief pressure applications(10-100msec) of isosmotic Na^+ solution were delivered to the soma from small (1 μm tip) pipettes appx. 100 msec before each 50 msec voltage jump. Since the flow of Na^+ solution into the choline solution was visually apparent, the area of membrane exposed to the sodium perfusion could be monitored and limited to 5-10 μm zones. Patch electrodes contained cesium which blocked outward currents. Voltage and current outputs from a switching single electrode voltage clamp (Axon Instru.) in the presence and absence of Na^+ were digitized, averaged and subtracted to obtain Na^+ currents. Temperature was 25°C.

On days 1-2, cesium-loaded neurons had input resistances of 3-5 GΩ. From a holding potential(Vh) of -80 mV, voltage jumps to -40 mV or more positive activated a fast inward Na^+ current. The current-voltage plots of peak currents were U-shaped with maximum currents at -10 mV. The time constant of decay of the Na^+ current during sustained depolarization was voltage-dependent(appx. e-fold decrease/10 mV depolarization). Steady state inactivation curves, h_∞ (V), were S-shaped with $h_\infty = 1$ at -100 mV and $h_\infty = 0$ at -40 mV. After 3-5 days in culture, the Na^+ current at the soma had a larger amplitude. Neurites became more prominent, and neuritic Na^+ currents could be recorded at the soma after focal application of Na^+ to individual neurites.

These results demonstrate that even early in development mammalian spinal cord neurons have inward Na^+ currents with kinetic behavior similar to that observed in other preparations. Comparison with "mature" cultured neurons should help in understanding the changes in spiking behavior that occur during development.

276.13 **CHLORIDE CHANNELS ACTIVATED BY GLUTAMATE AND GABA IN PATCH-CLAMPED ADULT XENOPUS LAEVIS NEURONS IN LONG-TERM CELL CULTURE.** D.M. Wetzel, S.D. Erulkar, L. Kilgren,* J. Rendt,* T. Parsons, and S. Yang,* Dept. of Pharmacology, Univ. of Pennsylvania, Philadelphia, Pa. 19104.

Glutamate and γ-aminobutyric acid (GABA) are major neurotransmitters (NT) in the amphibian spinal cord (SC) and brainstem (B) (Nistri & Constanti, Prog. Neurob. 13:117, 1979). GABA acts on motor neurons and spinal afferents as an inhibitory NT by hyperpolarizing the membrane through an increase in Cl^- conductance. Glutamate acts as an excitatory NT, causing membrane depolarization by increasing conductance to cations, primarily Na^+ and Ca^{++}. To examine these effects at the single channel level, cell culture procedures were developed which allow for the patch clamping of adult SC and B neurons maintained in cell culture. The B and SC of an adult male Xenopus laevis were removed while immersed in a well-oxygenated, chilled (4°C), normal frog Ringers solution (NFRS). The B and SC were minced into small pieces and transferred to a zero calcium-zero magnesium EDTA solution (NCM) for a 15-min. period. NCM was pipetted off and 1.25 mg/ml trypsin (1:250) in oxygenated NFRS at 4°C was added for a 1/2 hour incubation at 4°C. Following incubation, the B and SC pieces were triturated 100 x through a pasteur pipet, centrifuged (1,200 rpm) for 15 min, decanted, and then resuspended with trituration (50 x) in media without fetal calf serum (50% L-15, 5 mM D-glucose, 50 μg/ml gentamicin). Suspended cells were plated at low-density onto Falcon 3001 dishes previously treated with poly-l-lysine (400,000 mw). Media was as above, with addition of 5% fetal calf serum (Gibco FBS 309). Dishes were maintained in a normal atmosphere high-humidity incubator at 23°C ± 1°C for up to 6 months with occasional feedings at 2-month intervals. Neurons attached to the dish after 10-14 days and were most easily patch-clamped at 4-8 wks. Cell-attached, outside-out, and inside-out configurations of the patch clamp were used with various combinations of bath and electrode solutions. In the presence of 75 μM glutamate, single channels with a conductance of 30-40 pS were recorded. In low-sodium Ringer solutions, a glutamate-activated channel was found to be Cl^- conducting with a conductance of 150-206 pS. Open-time histograms fit with at least two exponentials with τ^1 = 1.5 - 1.9 mS and τ^2 = 6.6 - 11.8 mS. In the presence of 50 μM GABA channels with conductances of 80-100 pS were recorded. These channels appeared to be primarily Cl^- conducting. Open-time histograms plotted with three exponentials, τ^1 - 2.4 - 3.0 mS, τ^2 = 13-18 mS, and τ^3 = 45-160 mS. The responses to GABA and glutamate were seen in the majority (> 90%) of patches (N = 53). (Supported by NS02211, MH 46554-06, and BRSG S07-RR-05415-22).

277.1 PYRAMIDAL CELL DENDRITIC SPINE DEVELOPMENT IN LEAD INTOXICATED KITTENS. G. W. Patrick. Indiana University Sch. of Medicine, Dept. of Anatomy. Fort Wayne, IN. 46805

Lead has been suspected as a cause of several neurological disorders in children such as mental retardation, hyperactivity and autism, but underlying neuronal changes responsible for such sequelae have yet to be elucidated. This study was designed to determine the morphological consequences of relatively low doses of lead on structures associated with neuronal synapses. Beginning the day after parturtion kittens were dosed with 20 mg/kg lead acetate or 20 mg/kg sodium acetate by esophageal intubation. The animals were killed at weekly intervals from 1-5 weeks of age by an i.p. injection of pentobarbital. They were heparinized and perfused transaortically with 0.9% saline containing 0.1% procaine. The brain was quickly removed and placed in Golgi-Cox solution. After an appropriate staining time, blocked brains were embedded in celloidin and sectioned at 100 µm. Secondary branches of pyramidal cells in anterior sylvian, ectosylvian or suprasylvian gyri were selected for analysis. Spines were counted at 1,875X magnification for 27.5 µm of secondary dendritic length. The data presented here represents the visible dendritic spines. Beginning at 1 week sodium treated kittens show a steady increase in the number of spines per unit length. The average number of spines at 1 week was 18 rising to 19, 26, 29, and 35 spines for 2, 3, 4, and 5 weeks, respectively. Dendritic spine counts from lead treated kittens were 23, 33, 32, 36, and 50 for 1, 2, 3, 4 and 5 week-old animals, respectively. This data clearly demonstrates a dramatic increase in spine population ranging from 23-73% at different ages. The dendritic spines might disrupt temporal and spatial aspects of synaptic formation or may represent transient synapses which have failed to disappear, ultimately altering electrical activity of the pyramidal cell. These results thus suggest several mechanisms by which lead might produce neurological deficits.

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277.2 MPTP DESTROYS DOPAMINE NEURONS IN CULTURED RAT MIDBRAIN; MONOAMINE OXIDASE INHIBITORS PROTECT. C. Mytilineou and G. Cohen, Dept. of Neurology, Mount Sinai School of Medicine, New York, NY 10029.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin that destroys selectively the dopamine (DA) neurons of the substantia nigra in monkeys and man. We examined the effect of MPTP applied in vitro on the DA neurons of cultured rat midbrain. Explants of embryonic rat ventral midbrain were grown in organ culture. MPTP (10 uM) was added to the feeding medium for 4 or 7 days and the effect on the DA neurons was studied by catecholamine (CA) histofluorescence and ^{3}H-DA uptake. For histofluorescence, the cultures were incubated with alpha-methyl-norepinephrine in order to visualize all dopaminergic perikarya and processes. MPTP treatment resulted in a marked decrease in the number of fluorescing perikarya and nerve fibers. The effect was more pronounced after 7 days of treatment. MPTP also reduced the ^{3}H-DA uptake by the treated cultures to 61% of control at 4 days, and to 26% of control at 7 days. Pretreatment with 10 uM pargyline or 10 uM deprenyl, two monoamine oxidase inhibitors, protected the cultures from the neurotoxic effect of MPTP. ^{3}H-DA uptake by the pargyline-MPTP treated cultures was 58% of control compared to 15% of control in cultures treated with MPTP alone. Deprenyl completely prevented the toxic action of MPTP. CA histofluorescence confirmed the survival of DA axons after treatment with MPTP in the presence of monoamine oxidase inhibitors. Our results implicate monoamine oxidase in the neurotoxic effect of MPTP.

Supported by NIH grants NS-11631 and NS-18979 and by the American Parkinson Disease Association.

277.3 INORGANIC MERCURY IS A POTENT REVERSIBLE INHIBITOR OF GLUTAMATE UPTAKE IN ASTROCYTE CULTURES. N. Brookes, Dept. of Pharmacology & Exptl. Ther., Univ. of Maryland School of Medicine, Baltimore, MD 21201.

Primary cell cultures of astrocytes, derived from the cerebral hemispheres of newborn mice, possess an avid uptake mechanism for glutamate (Hertz, L. et al., Neurochem. Res., 3:1-14, 1978). In the present study, initial rates of [^{3}H] glutamate uptake in mouse astrocyte cultures at 2-3 weeks were measured during 1-8 min incubation periods in a Tris (20 mM)-buffered saline (pH 7.3, 35°C). Uptake was Na^+-dependent and followed Michaelis-Menten kinetics. The K_m was 98 ± 8 µM (SEM, n=4) and V_{max} was 69 ± 6 nmol/min/mg protein. Pretreatment of the cultures with dibutyryl cAMP 0.1 mM during the week before uptake measurement led to morphological differentiation of the astrocytes and a reduction of the K_m to 59 ± 2 µM (n=2) without significant effect on V_{max}. These results conform quite well with the findings of Hertz et al.

The influence of a number of inorganic metal ions on glutamate uptake in morphologically undifferentiated cultures was tested by addition of 1 µM chloride salt to the incubation solution, following a 10 min pre-equilibration of the cultures in buffered saline containing the same concentration of the metal chloride. Cobalt, strontium, lead, aluminum, cadmium and zinc chlorides (1 µM) were all without influence on glutamate uptake in solutions containing 50 µM and 2 mM glutamate. However, mercuric chloride 1 µM caused a marked inhibition of uptake at both glutamate concentrations. The concentration-effect curve for Hg^{++} was steep. Uptake was 14% inhibited in the presence of 0.2 µM Hg^{++}, 54% at 0.5 µM Hg^{++} and 75% at 1 µM Hg^{++} (glutamate 100 µM). Uptake was not different from controls in cultures exposed to 0.5 µM Hg^{++} for 10 min and then washed in buffered saline for 10 min prior to uptake measurement. The K_m for uptake in the presence of 1 µM Hg^{++} was not increased (66 ± 4 µM (n=2)), and preliminary data indicate a reduction in V_{max}.

Chronic exposure to Hg is associated with a multitude of neuropathological changes. The potent reversible inhibition of glutamate uptake by inorganic Hg reported here could provide a basis for understanding the reversible syndrome resembling amyotrophic lateral sclerosis (ALS) observed in a man acutely exposed to elemental Hg (Adams, C.R. et al., JAMA, 250:642-643, 1983) and indicates possible directions for research on the etiology of ALS. (Supported by U.S. Army contract DAMD 17-81-C-1279).

277.4 EFFECTS OF INTRASTRIATAL QUINOLINIC ACID INJECTIONS ON SEROTONERGIC AND DOPAMINERGIC NEURONAL SYSTEMS. S. Mazzari, C. Aldinio*, G. Toffano and R. Schwarcz+. Department of Biochemistry, Fidia Research Laboratories, 35031 Abano Terme, Italy and +Maryland Psychiatric Research Center, Baltimore, MD 21228, USA.

Following intrastriatal injection, the endogenous excitotoxic amino acid quinolinic acid (QUIN) causes selective "axon-sparing" lesions, which are reminiscent of those observed in the human neurodegenerative disorder Huntington's disease (Science, 219: 317, 1983). We have now examined the effects of unilateral intrastriatal injections of 300 nmol QUIN in rats on dopaminergic and serotonergic striatal nerve terminals. Dopamine (DA), serotonin (5HT), homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5HIAA) levels were measured 90min, 6hr, 4 and 11 days following QUIN administration. DA and 5HT turnovers were evaluated by measuring the accumulation of dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5HTP) 30 min after the administration of the aromatic amino acid decarboxylase inhibitor NSD 1015 (100 mg/kg, i.p.). Modifications in the injected striatum are listed as percent changes with respect to PBS injected controls (ND = no difference):

	90 min	6 hr	4 days	11 days
DA	ND	+ 24	+ 24	+ 24
HVA	ND	+ 21	+ 88	+ 36
DOPAC	ND	+ 57	+208	+ 78
5HT	ND	ND	ND	ND
5HIAA	ND	+ 77	+145	+ 41

Direct measurement of DA and 5HT turnover confirmed an enhanced activity in both afferent fiber systems 4 days following QUIN: after NSD 1015 treatment, both DOPA (+44%) and 5HTP (+42%) accumulation were significantly increased. No significant changes were noted in contralateral striata.

Based on the time curve of the observed effects, enhanced DA and 5HT turnover does not appear to be directly related to the excitatory actions of QUIN. Rather, the alterations reported here could constitute transient reactions of striatal monoaminergic afferents to the degeneration of their post-synaptic neuronal targets.

Supported by USPHS grant NS 16102 (to R.S.).

277.5 STUDIES ON THE CONTENT, SYNTHESIS AND DISPOSITION OF QUINOLINIC ACID IN THE RAT BRAIN. F.Moroni, G.Lombardi*, V.Carlà* Dept. of Pharmacology, Univ. of Florence, Viale Morgagni 65, 50134 Firenze, Italy.

Quinolinic acid (2,3 pyridinedicarboxylic acid, QUIN) is a tryptophan metabolite identified 35 years ago in mammalian liver and kidney. Recently QUIN has been added to the list of the excitotoxins because, when locally applied, it causes neuronal degeneration (Schwarcz et al.,Science 219,36,1983). We identified QUIN in the brain (Moroni et al.,Brain Res.,295, 352,1984) and we are now attempting to ascertain: a) whether or not the brain is able to synthetize it; b) whether or not it is possible to evaluate its turnover rate. QUIN was measured, as previously described, using mass spectrometry (single ion recording on m/z 272 and 2,4 pyridinedicarboxylic acid as an internal standard). When brain homogenates are incubated in phosphate buffer in the presence of tryptophan (10^{-4}M; 1h; 37°C),their content of QUIN increases at a rate of 0.60 nmol/g tissue/h. Tryptophan administration (200 mg/Kg i.p.) to adult rats increases not only the cortical content of 5HT (from 1.2±0.1 to 1.8±0.12 nmol/g tissue) but also the cortical content of QUIN (from 2.1±0.2 to 3.8±0.3 nmol/g tissue). These experiments indicate that the brain synthetizes QUIN. In order to measure its rate of synthesis, we attempt to antagonize its transport from the CSF to the blood by administering large doses of probenecid (200 mg/kg). This treatment linearly increases the cortical content of QUIN for at least 1h. Using the method of Neff et al. (J.P.E.T. 158, 214,1967) the evaluated rate of synthesis of QUIN was 0.7 nmol/g tissue/h. In the same cortical samples the rate of synthesis of 5HT was 0.36 nmol/g tissue/h.Thus QUIN seems to have a larger turnover rate than 5HT. In animals,whose brain tryptophan content is chronically increased (rats bearing for 4 weeks an anasthomosis between the porta and the cava veins), the cortical content of QUIN increased by 95%.

In conclusion, our data suggest that the brain synthetizes QUIN from tryptophan,that it disposes of it using probenecid sensitive transport mechanisms and that in pathological conditions the content of QUIN in brain increases.

Grants from CNR and the University of Firenze.

277.6 BRAIN ANOXIA OR BREAKDOWN OF MEMBRANE IONIC GRADIENT RELEASES NEURONAL STORES OF DOPAMINE IN THE RAT. J. A. Clemens, L. A. Phebus*, R. W. Fuller and K. W. Perry*. The Lilly Research Laboratories, A Division of Eli Lilly and Co., Indianapolis, IN 46285

In in vivo voltammetry experiments with carbon paste electrodes in anesthetized rats, we observed that when rats died during the experiment a large increment appeared in the first voltammetric peak (+0.12 V). Since dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and ascorbic acid (AA) all are components of the first peak, the identity of the substance was unclear. In this study in vivo brain dialysis was used in conjunction with in vivo voltammetry to identify the electroactive substance released after death.

Adult male rats were anesthetized, placed into a stereotaxic instrument and ventilated with a respirator. A miniature dialysis probe was lowered into the right corpus striatum and a graphite paste-tipped miniature working electrode placed into the left striatum. In vivo electrochemical measurements were made by clamping the potential of the working electrode vs the Ag/AgCl reference at a voltage positive enough to oxidize DA, DOPAC and AA but not other electroactive molecules. The oxidation current was continuously monitored on a strip chart. Saline was perfused through the dialysis tubing and DA, DOPAC, HVA, 5-HIAA, AA and 5-HT levels in the dialysate were monitored by HPLC. After a stable electrochemical baseline was achieved the respirator was turned off and the rat allowed to die.

At about 6 min following cessation of mechanical respiration, there was a large increase in dialysate DA concentration and in the electrochemical signal. AA, DOPAC, HVA and 5-HIAA levels in the dialysate declined. No 5-HT release was detected. In a separate experiment, perfusion of 10^{-3} M ouabain through the dialysis probe to inhibit Na/K ATPase resulted in a similar large increase in dopamine release. Both cerebral anoxia and ouabain are known to destroy membrane ionic gradients. This results in the high levels of extracellular dopamine. Similar high levels of dopamine have been reported to be cytotoxic. Thus, in pathological situations characterized by decreased blood flow or blood oxygenation such as stroke, ischemia or respiratory insufficiency DA may be released in quantities sufficient to be cytotoxic, such as through the generation of reactive free radicals.

277.7 INHIBITION OF SYNAPTOSOMAL GABA UPTAKE BY LIPID PEROXIDATION AND Ca^{+2}: ANTIOXIDANT EFFECTS OF GLUCOCORTICOIDS. J.M. Braughler, (SPON: G.D.Vogelsang) CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

The uptake of ^{14}C-GABA by rat brain synaptosomes was determined at 37°C in 200 µl of Krebs buffer, pH 7.4 containing 0.5 mg synaptosomal protein/ml and 10 µM GABA (1mCi/mole). GABA uptake was terminated after 5 min by the addition of ice cold Krebs followed by immediate filtration. Preincubation of synaptosomes with the free radical generators 100 µM xanthine and 0.3 units/ml xanthine oxidase (X/XO), or 0.1 mM H_2O_2 and 0.2 mM $FeCl_2$ (H/Fe) resulted in a temperature- and time-dependent inhibition of GABA uptake that was associated with formation of malonyldialdehyde (MDA). The effects of X/XO or H/Fe on GABA uptake and MDA formation were nearly completely blocked by catalase; were partially blocked by mannitol; and the effects of X/XO were enhanced by superoxidase dismutase, all suggesting that hydroxyl radical was involved. X/XO or H/Fe increased the Km for GABA from 6.8 µM to 19.8 µM and decreased the Vmax from 2.5 to 1.6 nmoles GABA/mg protein/5 min.

Preincubation of synaptosomes with the Ca^{+2} ionophore, A23187 (10 µM), caused a striking Ca^{+2}-dependent reduction in GABA uptake, and in addition, enhanced the inhibition of GABA uptake by X/XO or H/Fe. Ca^{+2} alone enhanced the actions of X/XO or H/Fe on GABA uptake which were less in Ca^{+2}-free Krebs than in Krebs containing 1.5 mM Ca^{+2}. The Ca^{+2} effects were blocked by 10 µM verapamil, but were not blocked by 10 µM indomethacin or 10 µM meclofenamate.

The inhibition of GABA uptake by X/XO or H/Fe could be prevented by preincubation (10 min at 37°C) of synaptosomes at 10 mg protein/ml with 100 µM methylprednisolone sodium succinate (MPSS) or 1 mM prednisolone SS (PSS). The effects of MPSS and PSS were concentration dependent and MPSS displayed a biphasic dose-response curve reminiscent of its dose-response characteristics in the injured cat spinal cord (J.M. Braughler and E.D. Hall, J. Neurosurg. 59:256-261, 1983). In contrast to MPSS or PSS, hydrocortisone SS at concentrations as high as 3 mM did not block lipid peroxidation-induced inhibition of GABA uptake. Steroids which blocked X/XO or H/Fe inhibition of GABA uptake also blocked MDA formation.

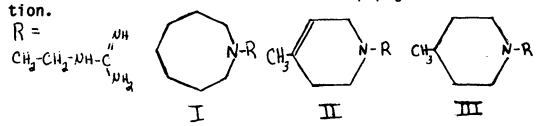
The results indicate that GABA uptake by synaptosomes can be inhibited by lipid peroxidation and a process involving Ca^{+2}. The glucocorticoids MPSS and PSS blocked the inhibition of GABA uptake by an antioxidant mechanism.

277.8 OCCUPATIONAL LEAD EXPOSURE: EFFECTS ON INFORMATION PROCESSING A.M. Williamson* and R.K.C. Teo*. (SPON. G.A.R. Johnston) Dept. Industrial Relations, Div. Occupational Health, Lidcombe N.S.W. Australia, 2141.

In a study of 59 lead workers, none of whom had ever had blood-lead levels above 80µg/10ml (mean of 49.56µg/100 ml) significant impairments were found using a number of tests from a battery designed around information processing principles. Discriminant analysis showed that these lead workers could be reliably differentiated from a similar group of 70 non-exposed controls on the basis of their generally lowered arousal levels, their greater fatigue in psychomotor tasks, their poorer short and long term memory as well as their tendency to trade off accuracy at the cost of speed in a prolonged visual tracking (vigilance) task. The degree of these deficits did not vary proportionately with amount of exposure except in the vigilance task which showed a significant relationship between behaviour and exposure. These results are consistent with the neuroanatomical findings of selective deposition of lead in the hippocampus of developing organisms and that lead is thought to produce segmental demyelination of peripheral nerves in both developing and adult organisms. Low-level lead exposure therefore affects specific aspects of information processing which are indicative of identifiable sites of damage to the nervous system.

277.9 GUANACLINE INDUCED ACCUMULATION OF FLUORESCENT LIPOPIGMENT IN SYMPATHETIC NEURONS. M.A. Palmatier,* P.T. Manning, R.E. Schmidt, P.A . Lampe* and E.M. Johnson, Jr. Wash. U. Med. School, St. Louis, MO 63110

Guanacline (II), an analog of the antihypertensive drug guanethidine (I), was tested clinically in the late 1960's. Clinical reports demonstrated markedly impaired sympathetic function up to 18 months after cessation of treatment. Sympathetic neurons of animals treated with guanacline accumulate fluorescent osmiophillic granules in a time- and dose-dependent manner. This lipopigment occurs in all species tested. Treatment of animals with either guanethidine or the saturated form of guanacline (III) does not cause fluorescent lipopigment accumulation.



I II III

The failure of these compounds to cause lipopigment is not due to failure of these compounds to accumulate in sympathetic neurons. At the EM level, the lipopigment granules initially contain multilamellar circular figures which vary in size but not morphology from species to species. Cessation of guanacline administration does not result in loss of lipopigment granules. Over time the lipopigment becomes granular with curvilinear shapes and increased fluorescence. Preliminary experiments show dissociated rat sympathetic neurons in culture will accumulate osmiophillic lipopigment in the presence of guanacline. Experiments are in progress to determine if this lipopigment is fluorescent and is caused by guanethidine or the saturated form of guanacline. These studies show that the double bond is critical for lipopigment accumulation and that sympathectomy and lipopigment accumulation occur by different mechanisms. We propose that the long-term sympathetic impairment seen in humans treated with guanacline, but not guanethidine, reflects functional correlates of the lipopigment accumulation or the process which produces it.

277.10 THE RELATIONSHIP BETWEEN NEUROLOGICAL DAMAGE AND NEUROTOXIC ESTERASE (NTE) INHIBITION IN RATS ACUTELY EXPOSED TO TRI-ORTHO-CRESYL PHOSPHATE (TOCP). B. Veronesi*+, S. Padilla*+, R. Novak*+, and L.W. Reiter. (SPON: Thomas Brock, III). +Northrop Services, Inc., Environmental Health Sciences and US EPA, Health Effects Research Laboratory, Research Triangle Park, NC 27711.

A rodent model of organophosphate-induced delayed neuropathy (OPIDN), which mimics the central and peripheral neuropathy seen in conventional chicken and cat models, has recently been described (Veronesi, B., Neuropath. Applied Neurobiol., 1984, in press). The present experiment was designed to relate neurochemical changes with neuropathy in rats acutely exposed to TOCP. A critical percent inhibition (>70%) of neurotoxic esterase (NTE), the putative target enzyme of neurotoxic organophosphates, has been shown to predict OPIDN in a variety of species exposed to various neurotoxic OP-compounds (Johnson, M.K., Rev. Biochem. Toxicol. 4:141, 1982). To determine if this relationship held in the rat model, seven groups of 70-90 d, Long Evans, male rats (n=3-12/treatment group) were exposed by gavage to acute dosages of TOCP (145-3480 mg/kg) or corn oil. All animals were prophylactically treated with atropine sulfate (7.5 mg/kg, s.c.) on the day of dosing and two days afterwards. Spinal cord and brain NTE activity, measured 44 hr post-exposure, was correlated with the appearance and degree of spinal cord damage in parallel groups of treated rats killed 14 d post-exposure. Our data indicate that dosages of TOCP that inhibit mean spinal cord NTE activity >72% of control values and mean brain NTE activity >66% of control values correlate with severe cervical cord degeneration in 95-100% of similarly dosed rats. In contrast, dosages that inhibit NTE activity less than these values produce only minimal spinal cord damage. These data suggest that in the rat, as in other species, a critical percent inhibition of NTE can be used to predict TOCP-induced neurological damage.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

277.11 DIFFERENCES AMONG INBRED MOUSE STRAINS IN MATERNAL AND FETAL SUSCEPTIBILITY TO TOXIC AND TERATOGENIC EFFECTS OF A SINGLE LARGE ORAL DOSE OF ETHANOL. R. Haddad and R. M. Dumas*. Neuroteratology Laboratory, New York State Office of Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Islans, NY 10314.

We have previously reported a single large oral dose of ethanol to be teratogenic to C57BL/6J mice but comparable doses given to CBA/J mice did not result in a significant incidence of malformations although an effect on fetal mass was detected in both strains (Haddad & Dumas. Teratogenicity of binge drinking: Comparative susceptibility of C57BL/6J and CBA/J mice to teratogenic effects of a single oral dose of ethanol. Alcoholism: Clinical and Experimental Research, 1982, 6:298).

We have subsequently compared the maternal toxicity and teratogenicity of a 5.8 g/kg dose of 25% ethanol given by gavage on gestation day (GD) 9 (vaginal plug = GD 1) in the following six inbred strains of mice: A/J, AKR/J, C3H/HeJ, CBA/J, C57BL/6J, DBA/2J. Statistically significant differences among the strains were found both in toxicity and in teratogenicity, but there was not a statistically significant relation between the proportion surviving the acute dose and the incidence of teratogenic effects in the surviving mice.

The DBA and AKR strains had a comparable acute maternal toxicity (approximately 15% on the day of treatment) but the DBA mice showed a significant incidence of teratogenic effects while the AKR strain did not. Since both of these strains fell within the same cluster in Taylor's analysis of the genetic similarity among 27 inbred strains of mice (B. A. Taylor, 1972, Genetic relationships between inbred strains of mice. J. Heredity., 63: 83-86) the implication is that the difference between them in susceptibility to the teratogenic effects of ethanol depends on relatively few genes. (We are indebted for this approach to Biddle's chapter entitled "The Role of Genetic Studies in Developmental Toxicology" in Developmental Toxicology, C. A. Kimmel & J. Buelke Sam, eds., Raven Press, 1981).

278.1 ACTIONS OF IDENTIFIED SINGLE SENSORY FIBERS ON DORSAL COLUMN NUCLEI NEURONS. D.G. Ferrington*, M.J. Rowe* and R.P.C. Tarvin*. (SPON: B.J. Williams). School of Physiology and Pharmacology, University of New South Wales, Sydney, Australia, 2033.

Many studies have demonstrated the high security of transmission within somatosensory pathways. In the present study in decerebrate or anesthetized [barbiturate +N_2O/O_2] cats, this has been investigated using paired recordings from single neurons in the gracile division of dorsal column nuclei and identified, intact sensory fibers of the interosseous nerve in the hindlimb. The sensory fibers were associated with Pacinian corpuscle (PC) receptors of the interosseous membrane and were activated by low amplitude (<5 μm) vibration at 200-300 Hz. Impulse activity recorded from the nerve was readily distinguished from background noise.

For fifteen gracile neurons it was possible to demonstrate that the neuron was driven by at least one fiber in the interosseous nerve. Almost all neurons studied responded with a high probability (greater than 90 percent) to the first spike in a series coming over a single PC input fiber. The mean latency of response was 9.8 ± 1.4 msec (n=19). Several neurons responded with pairs or triplets of spikes to the initial fiber spike and retained a high probability of response to the first 5-15 successive input spikes when the spikes arrived at 5 msec intervals.

Both convergence and divergence were observed in the input of interosseous nerve fibers to the gracile nucleus. Several neurons were influenced by more than one fiber. In one experiment a single fiber activated four neurons, three of which were studied in detail. The input fiber had a strong synaptic linkage with two of the cells and a weak linkage with the third.

The paired recording from identified PC sensory fibers and their target neurons of the gracile nucleus demonstrates directly the capacity of this somatosensory relay nucleus to respond reliably to minimal sensory inputs.

278.2 DISCRIMINATION OF THE FREQUENCY OF CUTANEOUS STIMULATION IS CRITICALLY DEPENDENT UPON THE DORSAL SPINAL COLUMNS. C. J. Vierck Jr. and R. H. Cohen. Dept. of Neuroscience, Col. of Medicine, Univ. of Florida, Gainesville, FL 32610.

After years of emphasis on spatial coding within the dorsal columns (DCs), it is now apparent that lateral pathways from the spinal dorsal horn can support excellent spatial resolution. Discriminations of spatial extent or of the relative positions of stimulated loci are impaired only transiently by DC lesions, and absolute localization or two-point discrimination are unimpaired. In contrast, tasks requiring discrimination of progressions of stimuli across the skin are severely and permanently impaired by DC interruption. These results suggest that the DC-lemniscal system uniquely codes spatiotemporal sequences of activity from sets of afferents successively stimulated by moving stimuli. However, before deciding that DC deficits are restricted to tests requiring an integration of spatial and temporal cues, capacities for purely temporal discriminations should be evaluated.

Four Macaca speciosa monkeys were trained to discriminate frequencies of stimulation of the sole of one foot. The stimuli consisted of 1 sec trains of half sinusoidal pulses of 11 msec duration and 550u excursion. Frequencies ranged from 10 Hz (the standard) to 35 Hz. Pulse durations and amplitudes remained constant, regardless of frequency, but the number of pulses and the interpulse interval changed. A signal detection paradigm permitted comparison of d′ values before and after surgery. Isolation of one DC by interruption of the ipsilateral dorsolateral column and the contralateral ventral quadrant produced no significant alteration in the discrimination of frequency. Interruption of the ipsilateral dorsal column produced a striking deficit (10 Hz could not be distinguished from 35 Hz) that was maintained for more than a year by two monkeys. It is concluded that fast conducting, quickly adapting, primary afferents in the dorsal columns are essential for fine temporal resolution of stimuli delivered to a population of cutaneous receptors. (Supported by PHS grants NS 07261 and MH 15737).

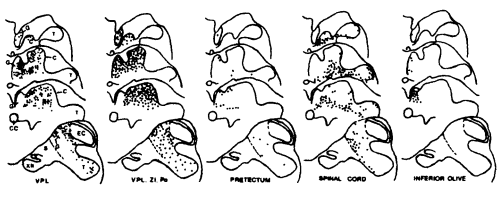
278.3 SUBDIVISIONS OF THE CAT DORSAL COLUMN NUCLEI BASED ON ITS EFFERENT PROJECTIONS. R.J. Budell* and K.J. Berkley. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306

Several anatomical studies have shown that the dorsal column nuclear complex (DCN) projects to a number of targets in addition to the ventroposterolateral nucleus of the thalamus (VPL). Although the data so far suggest that DCN neurons projecting to areas other than VPL are distinct from those projecting to VPL, little is known regarding the relative distribution of the extra-VPL-projecting populations. The present study was directed at this question.

Following single and double retrograde labeling strategies using appropriate combinations of ^{3}H-WGA, ^{3}H-apoHRP, WGA:HRP, fast blue and nuclear yellow, it was found that DCN neurons projecting to VPL, the spinal cord and the pretectum had different morphological features and were located in separable domains within DCN. Following increasingly larger injections of WGA:HRP into the diencephalon of different animals that extended outside of the boundaries of VPL to encroach upon the zona incerta (ZI) and posterior group (PO), it was found that while the total population of diencephalic-projecting neurons contained more small and fusiform neurons than the VPL-projecting population, the location of these small and fusiform neurons did not extend significantly into regions where spinal and pretectal-projecting populations were located. In all experiments, a small area of the gracile nucleus, just rostral to its clusters but caudal to the obex was virtually free of retrogradely labeled neurons. This region corresponds closely to the location of neurons projecting to the inferior olive (Molinari, 1984).

These results, summarized in the figure below, suggest that the populations of extra-VPL-projecting neurons occupy separable domains surrounding the region occupied by VPL-projecting neurons in DCN. Consequently, each population may constitute a functionally separate efferent system.

Supported by NIH grant NS 11892.



278.4 THE DISTRIBUTION OF TRIGEMINOCEREBELLAR, TRIGEMINOTECTAL AND TRIGEMINOTHALAMIC PROJECTION NEURONS: A DOUBLE RETROGRADE AXONAL TRACING STUDY IN THE MOUSE. D. A. Steindler, Anatomy Dept., University of Tennessee Center for the Health Sciences, Memphis, TN 38163

Divergent trigeminal projections to the thalamus, tectum, cerebellum, other brainstem nuclei, and spinal cord convey somatotopic representations of the head to these structures which are involved in processing of somatosensory responses and generating movements. Double retrograde axonal tracing has been used here to examine patterns of divergence in trigeminal projections to the cerebellum, superior colliculus and somatosensory thalamus. Paired injections of horseradish peroxidase and wheat germ agglutinin, N-[acetyl-^{3}H] were made in portions of the cerebellum, superior colliculus, and thalamic ventrobasal complex and/or posterior nuclear group of 20 adult ICR white mice anesthetized with intraperitoneal injections of Avertin. Sections through the brainstem trigeminal complex containing single and double retrograde neuronal labeling showed that: 1) Trigeminocerebellar (Tc) neurons are scattered amongst tectal (Tsc) or thalamic (Tth) projecting cells throughout the dorsoventral and mediolateral extents of the principal sensory and interpolaris divisions; 2) Large numbers of Tc cells occupy dorsal regions of the oralis division with fewer numbers of Tsc cells in more ventral regions, and only an occasional Tth cell can be found in oralis; 3) Tsc and Tth, but not Tc, projecting neurons reside in the magnocellular layer of the caudalis division, and Tth neurons were also found in the marginal layer; 4) Double labeled neurons were observed only after paired injections of the tracers in the tectum and ipsilateral thalamus, and these collateralized neurons were found within the principal sensory and the interpolaris divisions, in a ventral border region between the caudal principal sensory and rostral oralis divisions, and in the magnocellular layer of the caudalis division. Thus, there is at least one distinct class of Tc neurons without ascending projections and there may also be different classes of trigeminal neurons interrelating portions of the tectum and thalamus. There appear to be basic patterns of divergence in brainstem axonal projections whereby separate classes of neurons project to different combinations of target structures, and distinct intranuclear distributions of projection neurons reflect diverse patterns of innervation.

Supported by NIH Grant NS 15931.

278.5 FUNCTIONAL INFLUENCE OF THE ANTERIOR ECTOSYLVIAN SULCUS ON SOMATOSENSORY CELLS IN THE SUPERIOR COLLICULUS. H.R. Clemo and B.E. Stein. Dept. Physiology & Biophysics, Medical College of Virginia, Richmond, VA 23298.

A topographically organized somatosensory region, SIV, has been identified within the anterior ectosylvian sulcus (AES) (Clemo, H.R. and Stein, B.E, Brain Res., 235:162, 1982). SIV and its adjacent somatosensory region, para-SIV, send topographically organized inputs to the superior colliculus (SC) (Clemo, H.R. and Stein, B.E., J. Neurophysiol., 51:843, 1984). We have now examined the functional influence of this projection and found that its deactivation substantially altered the receptive field and response pattern of some SC cells and eliminated the responses of others.

The properties of 45 SC cells were studied in 11 paralyzed, anesthetized cats. Manual and electronically controlled stimulation of hairs, skin or deep tissue was conducted and optimal stimulus amplitudes and velocities were determined for each cell. The influence of the AES corticotectal projection was determined as follows: 1) after mapping the receptive field, the optimal stimulus was presented 10 times sucessively and the evoked discharges counted and displayed as rasters and histograms, 2) the AES was then cooled to <12°C and the same tests were repeated, and 3) the cortex was rewarmed and the same measures taken a third time.

Deactivating the AES depressed the responses of 55% of the SC cells activated by the gentle movement of hairs (n=29), 33% of those requiring displacement of the skin (n=7) and 14% of those requiring distortion of deep tissue (n=9). Thus, the majority (85%) of affected cells were 'hair' units and most (65%) had small receptive fields. The majority (60%) of those not affected were 'skin' or 'deep' units and most (66%) had receptive fields covering large portions of the body. The loss of normal corticotectal input from the AES was reflected as a significant (65-100%) decrement in the number of impulses evoked in affected SC cells. The magnitude of response depression varied in the receptive field and in some cells a portion of the receptive field became ineffective.

Apparently, the corticotectal input from the AES is submodality specific and its influence may vary throughout the receptive field of a given SC cell. This possibility is consistent with the manner in which the SC somatosensory receptive fields are constructed from converging afferents.

Supported by Grant NSF 8021559.

278.6 THALAMIC CONNECTIONS OF AREA 2 OF SOMATOSENSORY CORTEX OF MACAQUE MONKEYS: EVIDENCE FOR INPUTS FROM THE ANTERIOR PULVINAR AND SUBDIVISIONS OF THE VENTROPOSTERIOR NUCLEAR COMPLEX. T. P. Pons and J. H. Kaas. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

The thalamic connections of electrophysiologically defined parts of hand representations in somatosensory cortex of macaque monkeys (Macaca mulatta) were determined after separate or combined injections of wheat germ agglutinin (WGA) conjugated with horseradish peroxidase or tritiated WGA. After extensive microelectrode mapping of the hand representations in Areas 3b, 1, 2, and 5, and careful determination of the electrophysiological border between Areas 1 and 2, small injections of tracers were made in the representations of the glabrous portions of the digits in Areas 1 and 2. In all cases, connections were reciprocal.

Following injections into Area 1 (2 cases), as expected, the resulting thalamic label was located in the ventral half of the part of the ventroposterior nucleus representing the hand. No dense label was observed dorsally in the ventroposterior complex, and no label was seen in the anterior pulvinar.

In contrast, after injections restricted to the representation of the glabrous digits in Area 2 (3 cases), label was most dense in the anterior pulvinar (Pa) as well as in a dorsal portion of the ventroposterior complex that we have termed ventroposterior superior (VPS). Sparser label was seen more ventrally in the ventroposterior nucleus (VP) in two of these cases. Projections from Pa to Area 2 have not been previously reported.

In two additional cases where injections into the Area 2 hand representation also involved the Area 5 hand representation, label was seen in VP, VPS, and Pa. However, additional label was also seen in the lateral posterior nucleus.

We conclude that the hand representation in Area 2 of macaque monkeys receives major inputs from VPS and Pa, and a sparser input from VP. While much of the pulvinar is visual, projections to Area 2 implicate the anterior pulvinar in somatosensory functions.

Supported by NIH Grant NS16446.

278.7 SI AND SII PROJECTIONS FROM SOMATOSENSORY THALAMUS OF RATS. R. Weinberg, P. Barbaresi,* S. Cheema, R. Spreafico,* and A. Rustioni. Depts. of Anatomy and Physiology, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27514.

The topographic organization of the projections from the ventrobasal complex (VB) to the primary (SI) and secondary (SII) cortex represents an issue bearing on basic principles of thalamic organization and, more generally, as a clue to the significance of multiple cortical representations. Retrograde labeling approaches have been employed in cats to address this topic and, at the same time, to establish whether there exist thalamic neurons which project to SI and SII via branching axons. In the present experiments, which have been carried out in rats, the representation of the forelimb was first identified using standard electrophysiological techniques. The SII forelimb representation was located 1.4 mm above the rhinal sulcus in a zone that showed a vascular pattern similar to that described by Welker & Sinha (1972). In a first series of animals the physiologically-identified SII forelimb area was injected with WGA-HRP and processed using standard methods. In a second series, Fast Blue and Diamidino Yellow were injected into physiologically identified SI and SII forelimb representations and sections from the brains of these rats were examined under fluorescence microscopy. Cytoarchitectonic criteria and the pattern of commissural labeling were also used to verify the appropriate placement of the injections in SII. The focus of the injection was in a cortical region just above the rhinal sulcus characterized by a less dense layer IV than in SI. In the hemisphere contralateral to the injection site, retrogradely-labeled neurons and terminals of commissural fibers were found in an area exhibited by a less dense layer IV than in SI. Observations have so far focused on the results from the first series of experiments designed to identify SII-projecting thalamic neurons. In these cases, labeled neurons in the thalamus were concentrated in the posterior (PO) complex, but were present in VB as well. Within VB, the results so far collected suggest that SII-projecting neurons are concentrated in a posterior cap. Initial observations on the thalami with simultaneous injection of fluorescent dyes in SI and SII suggest that neurons with branching axons to both areas are relatively sparse, and, as expected from the single-injected animals, confined to the posterior part of VB.

Supported by USPHS grants NS 07132 and NS 16264.

278.8 CYTOCHROME OXIDASE STAINING REVEALS FUNCTIONAL ORGANIZATION OF MONKEY VENTRAL POSTERIOR THALAMIC NUCLEUS. E.G. Jones and S.H.C. Hendry, Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

In sections of the monkey thalamus stained histochemically for cytochrome oxidase, large, elongated clumps of reaction product can be detected in the ventral posterior medial (VPM) nucleus. Alternate sections were stained for horseradish peroxidase transported anterogradely from injections in the trigeminal nuclei, and immunocytochemically for glutamic acid decarboxylase or with the monoclonal antibody, CAT 301, in order to reveal co-localization of the three in the cytochrome oxidase positive clumps. Microelectrode recordings showed that all neurons recorded from in a clump responded to stimulation of the same or closely adjacent receptive fields. The clumps are, therefore, a morphological equivalent of the place and modality specific "rods" of cells that form the input to functional columns in the somatic sensory cortex (Jones et al., J. Neurophysiol. 48:545 (1982)).

The ventral posterior lateral (VPL) nucleus also stains densely for cytochrome oxidase. Although clumping is less obvious than in VPM of normal animals, the representations of individual peripheral nerves can be revealed by focal reductions in the staining subsequent to cutting the nerve. Cutting small cutaneous and/or deep nerves of the hand or foot leads to loss of stain in two or more elongated foci suggesting that multiple thalamic rods can receive inputs from the same peripheral nerve.

Supported by NIH Grant NS10526.

278.9 FACTORS DETERMINING PATCHY METABOLIC LABELING IN THE SOMATOSENSORY CORTEX OF CATS. S.L. Juliano, S.S. Cheema, B.L. Whitsel. Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27514.

Intermittent patches of metabolic label are set up in the somatosensory cortex by repetitive somatic stimuli. Although the distribution of label shifts systematically with changes in stimulus mode and location in a way which reflects known topographical and submodality gradients in the cortex, the intermittent and patchy quality of the labeling is less easily explained. One possibility is that the pattern reflects the selective activation of specific populations of mechanoreceptors. To test this possibility we carried out experiments using stimuli which non-preferentially activate all peripheral mechanoreceptors supplied by large diameter afferents. Such stimuli should enable assessment of the role of selective stimulation of mechanoreceptors in generating the cortical pattern. 5 cats received non-noxious electrocutaneous stimulation to the forelimb. The metabolic pattern obtained following IV injection of 14C-2deoxyglucose (2DG) was similar to that obtained with natural stimuli and also organized into well-defined patches. This finding suggests that preferential mechanoreceptor activation is not a major determinant of the intermittent somatosensory cortical labeling pattern.

To identify central factors which might underlie the patchy labeling pattern, experiments were initiated in which bicuculline (BIC) was applied to the somatosensory cortex of cats prior to IV injection of 2DG; these animals received bilateral electrocutaneous stimulation during the 2DG experiment. During these experiments, cerebral cortical electrical activity was monitored by recording EEG as well as single and multiple unit activity in SI. BIC was found to alter the 2DG pattern in 2 ways. At higher concentrations, the SI labeling occurred as (i) abnormally large (1.5 mm in tangential width) patches (ii) embedded in a homogeneous field of increased cortical 2DG uptake. At lower concentrations, the 2DG labeling also occurred as wide (1.5 mm) patches, but they were not embedded in a homogeneous field of increased 2DG uptake. The untreated hemispheres contained intermittent patches of fluctuating metabolic activity which ranged between 450-500 um in tangential width. These results indicate a role for intracortical processes in the formation of stimulus related metabolic patches. Supported by NS-10865, NS-07128 & DOD-N00014-83-K-0387.

278.10 SI CORTICAL NEURONS ARE INSENSITIVE TO THE SPATIAL FREQUENCY OF GRATINGS ROTATED ACROSS THE SKIN. S. Warren, H. Hamalainen, and E.P. Gardner. NYU Sch. Med., NY, NY 10016.

The perception of texture by the hand requires relative motion of surfaces across the skin. To assess the role of SI cortex in texture coding, we rotated wheels with surface-etched gratings (spatial periods, 0.8-9.6 mm) across the receptive fields of 49 SI neurons in alert monkeys.

29 neurons showed clear direction preferences in their responses (direction sensitive neurons (DSNs)): of these, 14 responded most vigorously to distal movements, 8 responded best to transverse motions towards the ulna, 3 preferred proximal motion, and 4 transverse radial motion. All DSNs responded with the same direction preference to textures of different spatial periods; in most, responses were independent of texture. However, 2 DSNs showed a monotonic effect of spatial period on average firing rates. One showed increased firing in all directions as the spacing between grating elements was increased; the other showed a similar increase in firing only when tested in the least preferred direction. Increasing the area of surface contact on the skin appeared to diminish the total discharge, particularly in the most preferred direction. The clearest differentiation of direction was observed with small diameter (0.625") and the worst with large diameter (2.5") wheels.

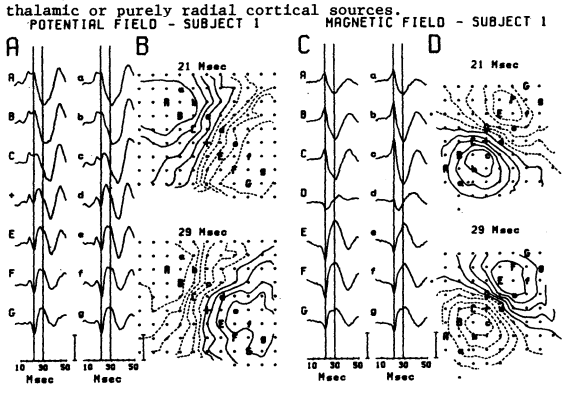
Motion sensitive neurons (N=12) responded equally to movements in longitudinal and transverse directions. Only 2 neurons showed linear changes in firing rate as a function of spatial period; no consistent effects of texture were seen in the others.

A new class of SI neurons, orientation sensitive neurons, (N=8) responded more vigorously to transverse than to longitudinal movements. Responses to motion in ulnar and radial directions were indistinguishable, and higher than to distal or proximal movements. None showed an effect of spatial period on firing rates.

We find the lack of texture sensitivity of SI neurons surprising because the quality of sensations evoked by rotating textured wheels across human skin depends upon spatial period. Fine textured wheels appear smooth, and evoke sensations of continuous motion. Rough textured wheels produce a punctate series of taps progressing linearly in a particular direction. The paucity of texture effects observed in our studies suggests that differentiation of surface texture does not occur in this population of SI neurons, but rather in some higher cortical area. (Supported by USPHS Grants NS11862 and NS17973).

278.11 COMPARISON OF MAGNETIC AND POTENTIAL FIELDS EVOKED BY HUMAN MEDIAN NERVE STIMULATION. C.C. Wood[1], D. Cohen[2]*, N. Cuffin[2]*, N. Yarita[3]*, T. Allison[1], and G. McCarthy[1]*. [1]VA Med. Cntr., West Haven, CT 06516 and Yale U., New Haven, CT 06520; [2]MIT, Cambridge, MA 02139; [3]Nihon Kohden Co., Tokyo, Japan.

Magnetic and potential fields produced by nervous system activity have different properties that can be exploited for the identification of electrical sources in the human brain. These include: (a) field orientations which differ by 90° for current dipole sources; (b) differential susceptibility to distortion by the dura, skull, and scalp; (c) differential sensitivity to radial and tangential sources; and (d) differential sensitivity to superficial and deep sources. We compared magnetic and potential fields occurring 20-35 msec following right median nerve stimulation in order to investigate the sources of evoked potential components attributed by different investigators to the thalamus or thalamic radiations, to a tangential source in somatosensory cortex, or to separate radial sources in somatosensory and motor cortex. The obtained magnetic and potential waveforms corresponded closely in morphology, with major peaks at virtually identical latencies. Their spatial fields were focally distributed over sensorimotor cortex, dipolar in shape, and differed in orientation by approximately 90°. These relationships are consistent with a tangentially oriented source in somatosensory cortex but are difficult to explain by either thalamic or purely radial cortical sources.



279.1 VERATRUM ALKALOIDS STIMULATE [^{3}H]-2 DEOXYGLUCOSE UPTAKE IN ASTROCYTES IN PRIMARY CULTURE. P. Yarowsky and N. Brookes, Dept. of Pharmacol. & Exp. Ther., Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

We have previously shown that the rate of glucose utilization in primary cultures of astrocytes from cerebral hemispheres of newborn mice is dependent upon the activity of the Na^+ pump. In unstimulated cultures (i.e., external [K^+] of 2.3 mM), 50-60% of the uptake and phosphorylation of 2-deoxyglucose (2-DG) was blocked by ouabain 1 mM or by reducing [K^+] to 0.44 mM or less (Yarowsky and Brookes, Soc. Neurosci. Abstr. 8:238, 1982). An influx of Na^+ induced by the Na^+/H^+ ionophore monensin (5 µM) augmented glucose utilization in astrocytes by over 3-fold and this response was totally eliminated by ouabain, and by Na^+-free or K^+- free solutions. It was unclear from these studies whether activation of a sodium conductance by other means would similarly increase the uptake of 2-DG in astrocytes. The membrane potential of astrocytes is insensitive to changes in external [Na^+] (Walz, et al., Brain Res. 292:367, 1984), but recently Bowman, et al. (Soc. Neurosci. Abstr. 9:448, 1983) have shown that the alkaloid veratridine induces a depolarization of the astrocyte membrane by activation of a tetrodotoxin-sensitive sodium conductance. Therefore, we sought to determine whether veratridine increases the uptake of 2-DG.

Primary cultures of astrocytes (14-22 days old) were incubated in Tris (20 mM)-buffered saline (pH 7.3) at 35°C with either veratridine (100 µM), veratrine (0.5 mg/ml) or protoveratrine A or B (100 µM) and the uptake of [^{3}H]2-DG was measured. Veratridine and veratrine increased the rate of glucose utilization by 74% and 77%, respectively, over control values while protoveratrines A and B were less effective, increasing uptake by 26% and 24%, respectively. The augmented uptake of 2-DG in veratrine-containing solutions was totally blocked by the addition of tetrodotoxin (3 µM), while control and monensin-stimulated uptakes were unaffected.

These results lend support to the existence of chemically-inducible, tetrodotoxin-sensitive Na^+ channels in the astrocyte membrane and further demonstrate the sensitivity of astrocyte energy metabolism to stimulation by influx of Na^+. (Supported by U.S. Army contract DAMD-17-81-C-1279 and NSF grant BNS 81-19481.)

279.2 KCl COTRANSPORT IS MORE IMPORTANT THAN NA^+-K^+ EXCHANGE IN THE CONTROL OF POTASSIUM CEILING LEVELS BY ASTROCYTES. W. Walz. Dept. of Pharmacology, Univ. of Saskatchewan, Saskatoon S7N OWO Canada.

In the normal brain activity-dependent fluctuations in the extracellular space (ECS) are limited to the range of 2-12 mM. The reason for this relative stability is the remarkable ability of glial cells to accumulate K^+ ions in excess of the 3 mM resting level. Astrocytes in primary cultures, equilibrated at 3 mM K^+, are capable of increasing their K^+ content from 520 to 900 nmol/mg protein within 50 s following an increase in external K^+ to 12 mM (cf. Walz & Hertz, J. Neurosci. Res. 10: 411, 1983). This phenomenon was analyzed in more detail. The equilibrated K^+ content at 3 mM K^+ was: 585 nmol/mg in a solution with normal ion composition, 630 nmol/mg after complete removal of all Cl^- ions and 380 nmol/mg after complete removal of all Na^+ ions. Exposure to ouabain (1 mM) led to a continuous reduction of the K^+ content in normal and Cl^- free solution (about 20 nmol x mg^{-1}/10 s), but had no effect in Na-free solution. Furosemide (2 mM) did not affect the K^+ content in any of these solutions. Exposure to 12 mM K^+ increased the K^+ content by 455 nmol/mg within 50 s; 233 nmol/mg of this accumulation were inhibited by ouabain application, 310 nmol/mg by furosemide application. Both drugs together inhibited 312 nmol/mg. Thus, the furosemide-sensitive component of the K^+ accumulation exceeded the ouabain-sensitive one by one-third. The effects are not additive, may be because both components can substitute each other to a certain extent. There is a third accumulation component which is resistant to both drugs. Passive K^+ movements are unlikely since 50 µM $BaCl_2$ which reduces K^+ permeability by 80%, had no effect on the accumulation. Removal of all Cl^- ions decreased the accumulation within the 50 s period from 455 to 270 nmol/mg. This component was furosemide-resistant. Removal of all Na^+ ions decreased the accumulation to 153 nmol/mg, 100 nmol/mg were furosemide-sensitive, ouabain had no effect. The furosemide-sensitive accumulation seems to be a KCl cotransport. Such a KCl shift together with water could be responsible for the observed shrinkage of the ECS in vivo. Although being an important component of the K^+ accumulation pathway, the Na^+-K^+ exchange seems to be more responsible in preventing loss of glial K^+. The results indicate that glial cells are responsible for volume control in the CNS during normal neuronal activity.

279.3 Na^+ AND K^+ CURRENTS IN RABBIT CULTURED SCHWANN CELLS. S.Y.Chiu*, P.Shrager and J.M.Ritchie*. Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06510.

Glial cells in peripheral and central nerves have long been thought to be inexcitable. We have used the patch-clamp technique to investigate membrane properties of Schwann cells cultured from sciatic nerves of newborn rabbits. Experiments were conducted with the whole-cell configuration after sealing the pipette tip to the cell surface and then rupturing the patch with suction. Resting potentials measured within 250msec after rupture were -30 to -40mV at 20C. The cell was then held at -75mV under voltage-clamp. A series of depolarising test pulses of increasing amplitude produced a family of ionic currents that appeared quite similar to those recorded from nerve fibers of many species. At potentials below +70mV there was a transient inward current followed by a steady outward current. Above +70mV the early transient current was outward. Addition of 4-aminopyridine resulted in complete block of the late outward current , with no effect on the inward current. The outward current was also absent when Cs^+ replaced K^+ in the patch pipette. The earlier transient current was blocked by tetrodotoxin, and was inactivated by brief prepulse depolarisations. We propose that these Schwann cells possess Na^+ and K^+ channels with many properties in common with those of nerve channels. If the patch pipette was withdrawn from the cell after whole-cell recording, an "outside-out" patch resulted from which we could record single channel currents. We have identified both Na^+ and K^+ channels. The density of Na^+ channels varied from 25 to 1000 channels per cell. Na^+ channels were also found in cultured Schwann cells from newborn rats, but their density was only about 10% that of the rabbit cells. We will discuss possible implications of these results for roles of Schwann cells in normal, remyelinating and regenerating neurons.
Supported by grants NS08304, NS12327 and NS17965 from the USPHS and by a grant RG1162 from the National MS Society.

279.4 INTEGRATION OF PERIPHERAL NERVE GRAFTS INTO GLIAL TOXIN-TREATED SPINAL CORDS. P.S. Fishman* and J.P. Kelly* (SPON: S. Max) Dept. of Neurology & the VA Res. Lab, Univ. of Maryland Sch. of Med., Balto., MD 21201, Dept. of Anatomy, Columbia Univ. Coll. of P. & S., NY, NY 10032

Although peripheral nerve (PN) grafts support the elongation of intrinsic spinal cord axons, these axons grow only a short distance upon re-entering the CNS. This phenomenon may be due to the poor penetration of graft Schwann cells (SCs) into the injured CNS, limited by reactive astrocytes (Fishman et al., Brain Res. 277:175, 1983). We wished to determine if the integration of PN graft derived tissue into injured spinal cords could be improved by pretreating host spinal cords with a substance toxic to astrocytes--6 aminonicotinamide (6AN). Blakemore has shown that 6AN causes glial cell death followed by remyelination of rat spinal axons by SCs. We injected 6AN (25-50mM, 1-5 ul) into the dorsal funiculi of the spinal cords of adult mice. Two to 7 days later a partial spinal cord transection was made at the previous injection site and a segment of autologous sciatic nerve was grafted between the severed spinal cord endings. Animals were sacrificed three to six weeks after graft placement, and the spinal cords were processed for immunohistochemistry as well as conventional histology. Animals with 6AN injections alone showed significant astrogliosis, with increased immunoreactivity with antisera against glial fibrillary acid (GFA). 6AN treated mice contained many SCs within the spinal cord. SC products were demonstrated with antisera against the peripheral myelin protein PO and the SC basement membrane protein laminin. SC antigens were in general localized to the dorsal root entry zones and the central grey matter. These regions were surrounded by highly reactive astrocytes but devoid of significant GFA immunoreactivity, forming separate astrocytic and SC domains within the spinal cord. 6AN treated plus PN grafted cords were also highly gliotic, but the interfaces of graft and host tissue were less prominent with GFA immunohistochemistry than those of grafted but untreated animals. SC products were found radiating out from the graft into the treated host spinal cord where they frequently merged with treatment related SC domains. Whether the presence of Schwann cells within the spinal cord can facilitate the ingrowth of graft derived axons remains to be tested.
Supported by the VA and The Bressler Res. Fund.

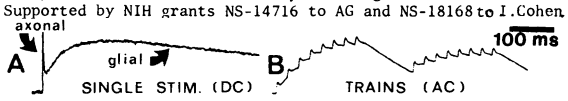
279.5 IS THERE A POTASSIUM DEPENDENT DEPOLARIZATION OF THE PARANODAL REGION OF MYELIN SHEATH? OPTICAL STUDIES OF RAT OPTIC NERVE. V.Lev-Ram* and A.Grinvald. Depts. of Cell Biology and Neurobiology, Weizmann Institute, Rehovot,Israel 76100.

Investigation of axon-glia interactions in myelinated nerve has been hampered by the difficulty of studying the membrane properties of the oligodendrocytes at the specialized interaction sites such as the paranodal region. The use of suitable fluorescent voltage-sensitive probes and an optical recording technique now permits investigation of the glial responses to axonal activity.

Rat optic nerves were maintained in oxygenated Krebs solution, and were incubated with 50-100μM of the fluorescent probe for 2-3 hours. (Extracellular recording indicated insignificant deterioration, for at least 6 hours.) When the stained nerve was stimulated with a suction electrode, electrical activity in the axonal membrane and potential changes in the supporting cells were recorded optically:Two types of optical signals were detected; a fast optical signal (3-5 msec) similar in time course to that expected for the sum of intracellular electrical recordings from the population of axons and a slow signal (latency to peak 40-90 msec, decay time 700-1300 msec) presumably from glia bound dye (See A below). The amplitude of the slow signal varied with extracellular potassium (1.2mM-11.2mM) and interstimulus interval in a manner characteristic of glial depolarization (See B below). The amplitude of the slow component increased when 4-AP (5mM) was applied for 6-20 min but, markedly decreased if the drug was applied for 4 hours. This is consistent with a location of the slow signal at the oligodendrocyte paranodal region. The slow component was absent in axons loaded with Cesium. Lowering temperature increased the relative amplitude of the slow signal and slowed its time course. Both fast and slow signals were abolished by TTX (10^{-6}M), except near the stimulating electrode, where passive spread could evoke the slow signal.

The results suggest that the slow signal is sensitive to potassium accumulation, it is of glial origin, and at least a portion of it originates in the paranodal region. This optical signal might be useful for the investigation of the early site of the lesion in demyelinating diseases.

Supported by NIH grants NS-14716 to AG and NS-18168 to I.Cohen.



279.6 SCHWANN-CELL RESPONSES DURING EARLY WALLERIAN DEGENERATION. A. L. Oaklander*, M. S. Miller, and P. S. Spencer. Institute of Neurotoxicology and Departments of Neuroscience and Pathology, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Schwann cells undergo profound alterations in phenotypic expression as a consequence of axonal degeneration. Myelin formation ceases and Schwann cells adopt a quiescent state within longitudinal columns delimited by basal lamina. The sequence and triggers of these events are unknown, although it has been suggested that loss of axon-Schwann cell apposition may be involved. This study examines early changes in synthesis of DNA, RNA, and protein in the distal stump of transected peripheral nerve. Activity of ornithine decarboxylase, required for polyamine synthesis and induced in differentiating cells, was also measured.

The right sciatic nerve of CD-1 mice was transected at the sciatic notch; the left thigh was sham-operated. Transected and control nerves were excised for incubation with radiolabelled precursors at 12-h intervals from 0-5 days post-transection. Seven animals were used at each timepoint. Sciatic nerves were removed, desheathed (perineurium + epineurium) and the most proximal millimeter of the distal stump discarded. Tissue was incubated at 37°C for 2 h in media containing ^{3}H-thymidine, ^{3}H-uridine, ^{14}C-ornithine, or a ^{14}C-amino acid mixture. Incorporation of radioisotope was linear up to 3 h. Following incubation, nerves were rinsed in saline, homogenized in 5% trichloroacetic acid (TCA) and centrifuged. Pellets were repeatedly washed with 5% TCA containing unlabelled precursors to remove unincorporated radioactivity. The TCA-insoluble pellets were dissolved and aliquots removed for liquid scintillation counting and protein assay.

Incorporation of ^{3}H-thymidine, a marker of the premitotic S-phase of the cell cycle, increased from sham-operated (control) values within 1.5 days post-transection and peaked at approximately 15X control values at 4 days post-transection. Incorporation of ^{3}H-uridine, a measure of total RNA synthesis, was elevated over control values at 1-2 days and again at 4-5 days. Incorporation of ^{14}C-amino acids into protein was increased over control values by 1.5 days, peaked at 2.5 days and remained elevated up to 5 days post-transection. Activity of ornithine decarboxylase was elevated in both transected and sham-operated nerves.

These data demonstrate that measurable alterations in Schwann-cell function occur within 36 hours of axonal transection, well before axon-Schwann cell apposition is lost. Loss of axonal contact may not be the sole mechanism triggering early Schwann cell responses during degeneration of peripheral nerves.

Supported by NS 19611 and Shell Companies Foundation.

279.7 DIFFERENTIATION OF ASTROCYTES IN VITRO FROM IMMATURE NEUROECTODERMAL CELLS. J.E. Goldman and S.S. Geier*. Dept. of Pathology (Neuropathology), Albert Einstein Coll. Med., Bronx, NY 10461.

Dissociated cell cultures, established from neonatal rat forebrain, have been used for a number of years to study properties of astrocytes, since the large majority of cells express glial fibrillary acidic protein (GFAP), an astrocyte characteristic, as assessed by immunocytochemical and metabolic labeling studies (Chiu and Goldman, J. Neurochem, 1984). Little GFAP is found in the forebrain of neonatal rats, however, suggesting that astrocytic differentiation may take place in culture. Until recently, it has not been possible to follow glial differentiation from immature cells, because, although cellular markers for mature glia are known, the molecular properties of undifferentiated neuroectodermal cells remain uncharacterized. We have recently identified GD3 ganglioside as a characteristic surface glycolipid of immature neuroectodermal cells in the rat CNS, cells localized to the subventricular zone in neonatal forebrain (J Goldman et al, J Neuroimmunol, in press).

We have used a double-label immunofluorescence method to follow the expression of GD3 and GFAP in cultures from neonatal rat forebrain, using a monoclonal antibody to GD3 (Pukel et al, J Exp Med, 1982), and a rabbit antiserum to GFAP. Initially 5-10% of cells are GD3+, while few express GFAP. Within the first few days in vitro, GFAP expression begins in GD3+ cells. After 7-10 days in vitro, many of the GFAP+ cells no longer bind the GD3 antibody. These kinetics suggest a developmental sequence, in which an astrocytic characteristic arises in a cell derived from the subventricular zone. In addition, a highly enriched population of GD3+ cells, produced by immunoadsorption, acquire GFAP when cultured in the presence of fetal calf serum (FCS). In cultures established at high cell density, small, process-bearing GD3+ cells grow on top of an astrocyte monolayer. When these cells are shaken off (McCarthy and deVellis, J Cell Biol, 1980), and cultured separately, the large majority acquire GFAP. We infer that conditions in vitro, including the presence of FCS, induces an astrocytic differentiation of immature cells. Steps in the acquisition of astrocytic properties and factors that induce such properties can be analyzed. Supported by USPHS Grant NS17125, and NS00524.

279.8 MODULATION OF BETA-ADRENERGIC RESPONSE IN RAT BRAIN ASTROCYTES BY SERUM AND HORMONES. D. K. Wu*, R. S. Morrison and J. de Vellis (SPON: T. L. Ritchie). Lab of Biomedical and Environmental Sciences, Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

Purified astrocyte cultures from neonatal rat cerebrum respond to beta-adrenergic agonists with a transient rise in cAMP production. This astroglial property was regulated by serum, a chemically defined medium (serum-free medium plus hydrocortisone, putrescine, prostaglandin $F_{2\alpha}$, insulin and fibroblast growth factor) and epidermal growth factor. Compared to astrocytes grown in serum-supplemented medium, astrocytes grown in the chemically defined medium were non-responsive to isoproterenol stimulation, and this difference did not appear to be due to selection of a subpopulation of cells by either medium. An active substance(s) in serum was responsible for restoring the responsiveness of astrocytes. Each of the five components of the defined medium had little effect by itself; however, together they acted synergistically to desensitize astrocytes to beta-adrenergic stimulation. On the other hand, epidermal growth factor, a potent mitogen for astrocytes, was very competent by itself in reducing the cAMP response of astrocytes to beta-adrenergic stimulation. The data suggest that a decreased beta-adrenergic receptor number and an enhanced phosphodiesterase activity may account for the reduced response to beta-adrenergic stimulation. Thus, purified astrocytes grown in the chemically defined medium appear to be a good model for the study of hormonal interactions and of serum factors which may modulate the beta-adrenergic response.

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279.9 THE RAT OPTIC NERVE FOLLOWING ENUCLEATION: A PURE PREPARATION OF MAMMALIAN GLIA. B.R. Ransom and C.L. Yamate*. Dept. of Neurol., Stanford Univ. Sch. of Med., Stanford, CA 94305.

Advances in understanding glial function have depended importantly on the exploitation of favorable preparations (e.g. leech ganglia, necturus optic nerve, etc.). Knowledge of mammalian glia has been partially frustrated by the lack of an exclusively glial preparation; a preparation devoid of neurons, axons, and synapses. We have found that the rat optic nerve (RON) following enucleation is well-suited for the electrophysiological analysis of mammalian glia uncomplicated by intermixed neural elements. Neonatal rat pups underwent unilateral enucleation using standard mechanical technique. After a survival period of at least 4 weeks, the optic nerves from both the normal and enucleated sides were dissected free, placed in a recording chamber, and perfused with Ringer's solution containing 5 mM $[K^+]$. All experiments were conducted at 37°C. The diameter of the enucleated RON was only 10-20% that of the normal RON and was translucent rather than white and opaque, the appearance of the normal adult RON. Standard intracellular recording techniques were employed in this study. As the electrode was advanced through the nerve inexcitable cells were encountered with high resting membrane potentials (average = -79 mV±6; N=27). When $[K^+]_o$ in the perfusate was altered from 5 to as high as 60 mM, these cells briskly depolarized and the average slope for a 10-fold change in $[K^+]_o$ was 49±7 mV (N=8). This is significantly less than the slope expected if $[K^+]_o$ were the sole determinant of resting membrane potential. While continuously recording from glial cells a variety of putative neurotransmitter substances were bath applied. Acetylcholine (10 mM) and adenosine (1 mM) were without effect. Glutamate depolarized glia in a dose-dependent fashion by as much as 10 mV at a concentration of 5 mM. GABA (up to 10 mM) produced small depolarizations in 20% of the cells tested. Further studies along these lines are in progress. These observations establish the enucleated rat optic nerve as a favorable preparation for studying mammalian glia in the absence of neuronal tissue. Supported by NIH grants NS 15589 and NS 00473 from the NINCDS.

279.10 MYELIN-ASSOCIATED GLYCOPROTEIN (MAG): IMMUNOCYTOCHEMICAL LOCALIZATION IN BOTH OLIGODENDROCYTES AND MYELIN LOOPS OF MYELIN DEFICIENT (MLD) MUTANT MICE. F. X. Omlin* and J.-M. Matthieu* (SPON: G. M. Innocenti). Institut d'Histologie et d'Embryologie de l'Université de Lausanne, CH-1011 Lausanne, Switzerland.

Myelin-associated glycoprotein (MAG) is a quantitatively minor membrane constituent which is believed to be involved in glia-axon interactions. Immunocytochemical studies indicate that MAG is localized in periaxonal regions, paranodal loops and Schmidt-Lantermann incisures (Trapp, B. D. and Quarles, R. H., J. Cell Biol., 92: 877, 1982). Recently, these observations have been challenged (Webster, H. deF. et al., J. Neurochem., 41: 1469, 1983). Since the CNS of myelin deficient (mld) mutant mice is characterized by a severe myelin deficit and a high frequency of both loose myelin lamellae and myelin loops, we used this model to gather additional information on the localization of MAG.

Optic nerves and spinal cords of 15-and 25-day-old mld and age-matched control mice were prepared for immunocytochemistry and electron microscopy. Both techniques revealed two categories of oligodendrocytes in mld mice. One class which corresponded to that present in controls, showed a granular pattern of narrow perinuclear immunostaining. The fine structure of these typical oligodendrocytes was indeed the same as in controls. The other class of mld oligodendrocytes which was absent in the controls, showed a very intense MAG immunostaining scattered over the whole large cytoplasmic area. The size of these immunostained granules was variable. At the electron microscopic level, these large cells displayed a dark cytoplasm which was completely filled with acuolar profiles of different sizes corresponding to the immunostained granules. Furthermore, these cells had an abnormal and extended endoplasmic reticulum, but a lack of recognizable Golgi apparatus. The number of necrotic cells was not increased in mld tissue when compared to the age-matched controls. Thin sections of mld tissue also showed extended and complex myelin loops. These loops were clearly immunostained for MAG. In conclusion, these results indicate that MAG is localized in uncompacted CNS myelin lamellae and extend recent investigations in the PNS (Trapp, B. D., et al., Trans. Am. Soc. Neurochem., 15: 239, 1984).

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279.11 KINETIC AND PHARMACOLOGIC CHARACTERIZATION OF THE GLUCOSE TRANSPORT SYSTEM IN C6 GLIOMA CELLS. W. Logan, A. Klip* and E. Gagalang*. Div. of Neurology, Research Institute, The Hospital for Sick Children and Univ. of Toronto, Toronto Ontario, Canada M5G 1X8.

The glucose transport system of C6 glioma cells was studied by measuring the uptake of the non-metabolizable sugars ^{3}H-3-O-methyl-D-glucose (MG) and ^{3}H-2-deoxy-D-glucose (2dG). Kinetic parameters of uptake can be measured with the former sugar, since it is not phosphorylated after transport. In cells grown in 2% fetal calf serum in MEM, saturable uptake of MG had a V_{max} of 2 nmol/min·mg cell protein and a K_m of 3 mM at room temperature. These values are in close agreement with those previously observed in L6 myoblasts. In contrast to MG, uptake of 2dG (0.1mM) was linear for periods longer than 1 min (up to 10 min), due to sugar phosphorylation and prevention of back-flux. The rate of 2dG uptake measured at 10 min was 145 pmol/mg·min compared to 120 pmol/mg·min for the uptake of MG at 10 sec. Therefore transport seems to be the limiting step in 2dG uptake at 10 min.

Uptake of either MG or 2dG was selectively inhibited by the mold metabolite cytochalasin B (CB) with a K_i of 5 x 10^{-8} M. Hexose uptake was virtually independent of extracellular Na^+ or K^+ (osmotically substituted by choline). Incubation in 10% fetal calf serum progressively stimulated uptake of 2dG or MG up to 100%. Platelet derived growth factor stimulated hexose uptake by 30% in 1 h. Insulin, on the other hand, had no effect on the rate of hexose uptake. In contrast to the behavior of other cells in culture such as L6 myoblasts and fibroblasts, cell density had no effect on the rate of sugar uptake by the glioma cells.

It is concluded that the glucose transport system of the C6 glioma cell is similar to that of other non-epithelial cells in culture in that it is saturable, rate-limiting for glucose accumulation and inhibited by CB. The hexose uptake system of these cells has retained certain regulatory mechanisms and may closely resemble that found in normal glial cells.

279.12 SYNTHESIS OF SULFATED GLYCOPROTEINS BY CULTURES OF PURIFIED OLIGODENDROCYTES AND ASTROCYTES. N.R. Bhat* and E.G. Brunngraber. Neurochem. Unit, Missouri Inst. Psychiatry, Dept. Biochem., Univ. Missouri-Columbia, Sch. Med., St. Louis, MO 63139

Previous studies on the analysis of brain glycoproteins have demonstrated the occurence of sulfated and nonsulfated sialoglycopeptides, a situation analogous to that of the two forms of the marker glycolipids of myelin and oligodendrocytes: cerebrosides and sulfatides. It is known that some of the myelin associated glycoproteins are sulfated. In order to evaluate the capacity of the myelin forming cells to synthesize sulfated glycoproteins, we used pure cultures of oligodendroglial cells derived from primary cultures of neonatal rat brain cells. The cells were labeled with ^{3}H-glucosamine and inorganic ^{35}S-sulfate. After the extraction of the cells for lipids the defatted residue was digested with pronase for three days with daily additions (1 mg/ml) of the protease. The digest was boiled for 10 min and centrifuged. The clear supernatant was desalted on a biogel P-2 column and made up to 0.04 M NaCl. The glycosaminoglycans were precipitated with cetyl pyridinium chloride. The supernatant was freed of the detergent by n-amylalcohol extraction before fractionation on a Con A-Sepharose affinity column. The glycopeptides were separated into the complex N-linked tri- and tetraantennary sialoglycopeptides and the O-linked glycopeptides (unbound), the biantennary (weakly bound) and high mannose (strongly bound) glycopeptides by eluting the column sequentially with the starting buffer, 20 mM and 200 mM α - D methyl mannoside. The N-linked complex type and O-linked oligosaccharides were separated from each other by gel filtration on a Sephadex G-50 column after mild alkaline hydrolysis. The O-linked oligosaccharides contained only 10-20% of the total ^{3}H-glucosamine label. Almost all of the ^{35}S label was contained in N-linked glycopeptides. The % distribution of the ^{3}H label among N-linked oligosaccharides was approximately 60, 30 and 10 in complex type, biantennary type, and high mannose type glycopeptides, respectively. On the other hand, greater than 80% of the ^{35}S label was associated with complex (tri- and tetraantennary) glycopeptides, the rest being associated with the biantennary type. The sulfation density (^{35}S/^{3}H) was also higher in the sialoglycopeptide fraction. Even though the distribution of the ^{3}H-glucosamine into glycopeptide fractions from oligodendrocytes and astrocytes was similar, the former showed markedly higher (4-5 times) rates of glycoprotein sulfation.

280.1 α_2-RECEPTOR CONTROL OF Ca^{++}-MEDIATED REDUCTION OF VOLTAGE-DEPENDENT K^+ CURRENTS. M. Sakakibara*, D.L. Alkon, I. Lederhendler*, and E. Heldman* (SPON: J.W. Daly). Section on Neural Systems, Lab. of Biophysics, NINCDS at MBL, Woods Hole, MA 02543.

J. Farley has recently shown (Soc. Neurosci. Abstr.,1983) that extracts of the Hermissenda optic ganglion increase Type B photoreceptor input resistance and the long-lasting depolarizing response (LLD) to light. In a previous neurochemical analysis of the optic ganglion (Heldman et al., J. Neurophysiol., 1979) we identified a single optic ganglion cell which reliably showed green fluorescence using the Falk-Hillarp method for determining the presence of catecholamines. To test the possibility that this cell might contribute to the effect of Farley's optic ganglion extract on the Type B cell we added separately dopamine ($\leq$ 500 µM) and the α_2-receptor agonist, clonidine ($\leq$ 300 µM), to the ASW perfusion medium. Dopamine had little or no effect while clonidine caused a marked increase of the Type B cell input resistance as well as in the steady-state depolarizing response to light. The LLD (after the light) also increased in magnitude and duration. Voltage clamp experiments with Type B somata (following axotomy to remove impulse activity and synaptic interactions) demonstrated that clonidine caused a marked reduction of the two voltage-dependent K^+ currents (but not the voltage-dependent Ca^{++} current) which comprise most of the outward current at potentials -60 to 0 mV: an early rapidly activating current, I_A, and a late Ca^{++}-dependent K^+ current, I_C. Both of these currents remain reduced on days after associative learning (cf. Forman et al., 1984, Soc. Neurosci. Abstr.). When 10 mM Ba^{++} was substituted for the 10 mM Ca^{++} in the external bathing medium, the clonidine-reduction of I_A was almost eliminated. Addition of yohimbine ($\leq$ 100 µM), an α_2-receptor antagonist, to ASW caused marked reduction of the intact Type B depolarization during and after a light step and eliminated EPSP's (under presynaptic control of the S/E optic ganglion cell) which enhance cumulative depolarization of the Type B cell during conditioning with repeated pairings of light and rotation. Yohimbine also reduced Ca^{++} mediated inactivation of I_C of isolated Type B somata. These results suggest that activation of α_2-receptors within the Type B membrane by a substance released from pre-synaptic optic ganglion cell(s) could amplify Ca^{++}-mediated I_A and I_C reduction, by which conditioning-activation of visual-vestibular neural systems encode and store a learned association of distinct sensory stimuli (cf. Alkon, J. Physiol., Paris, 1982-3).

280.2 α_1-ADRENOCEPTOR-EVOKED PACEMAKER ACTIVITY IN SEROTONERGIC DORSAL RAPHE NEURONS: ROLE OF INCREASED CALCIUM CONDUCTANCE. G. K. Aghajanian, Depts. of Psychiat. and Pharmacol., Yale Univ. Sch. of Med., Conn. Mental Health Ctr., 34 Park St., New Haven, CT, 06508.

Serotonergic (5-HT) dorsal raphe neurons have a slow, tonic pacemaker firing pattern in vivo; intracellular recordings in vivo show that spikes arise from depolarizing ramps, not discrete synaptic potentials (1). In brain slices, most viable 5-HT neurons are silent but pacemaker activity can be restored by norepinephrine or phenylephrine (PE), an α_1-agonist (2).

The ionic basis for α_1-induced pacemaker activity in 5-HT neurons was studied in a rat midbrain slice preparation. Upon impalement, 5-HT cells were initially activated, but as "sealing" progressed, membrane potentials (V_m) deepened to -60 to -80 mV, and most cells became silent. Hyperpolarizing pulses induced anode-break prepotentials at a V_m of -58 to -60 mV; these were blocked by Ca^{2+} antagonists but not TTX. In the presence of TTX, high-threshold Ca^{2+} spikes were also observed (cf., Ref.#3). At concentrations of PE too low to activate cells (e.g., 1-2 micromolar), there was a depolarization accompanied by an increase in R_{in}; reversal potentials (E_{rev}) were about -92 mV, indicating a decrease in g_K. At concentrations of PE which induced rapid spiking (e.g., 25 micromolar), a E_{rev} could not be obtained and R_{in} was not always increased. In TTX treated slices, when Na^+ spikes were blocked, high concentrations of PE enhanced both low and high-threshold Ca^{2+} potentials and markedly increased afterhyperpolarizations mediated by the Ca-activated g_K. It is concluded that α_1-adrenoceptor agonists evoke pacemaker activity in 5-HT neurons by two mechanisms: a decrease in "resting" g_K and an increase in voltage-dependant g_{Ca}.

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(2) VanderMaelen, C.P. and Aghajanian, G.K. Brain Res. 289: 109-119, 1983.
(3) Llinas, R. and Yarom, Y. J. Physiol. 315: 569-584, 1981.

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280.3 LONG-LASTING FACILITATION OF PEPTIDERGIC TRANSMISSION BY CATECHOLAMINES IN SYMPATHETIC NEURONS IS MEDIATED BY CYCLIC AMP. N. Mo*, Z.G. Jiang* and N.J. Dun (SPON: R.S. Schmidt), Dept. of Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL. 60153

Repetitive stimulation (10-20 Hz, 1-2 sec) of the hypogastric nerves evoked in neurons of the guinea pig inferior mesenteric ganglia, in addition to the fast excitatory postsynaptic potential (epsp), a non-cholinergic epsp the mediator of which has been proposed to be substance P or a related peptide. When applied to the ganglia in the concentrations of 10 µM or less for 3-5 min, epinephrine (EPI), isoproterenol (ISO) and norepinephrine (NE) caused an initial, short-lasting depression which was followed by a large augmentation of the non-cholinergic epsp lasting from minutes to over hours. Dopamine (DA) employed in the same concentration caused only a slight depression that was not followed by a noticeable increase of the non-cholinergic epsp. The α-agonists were more potent in depressing, while β-agonists being more effective in enhancing, respectively, the non-cholinergic transmission. The catecholamine-induced depression and subsequent enhancement of the non-cholinergic epsp was prevented by α-adrenergic antagonists (dihydroergotamine and phenoxybenzamine, 1-10 µM) and β-adrenergic antagonists (propranolol and dichlorisoproterenol, 10 µM), respectively. The membrane depolarization induced by the putative transmitter substance P (1 µM) was similarly augmented by ISO; this effect which could be blocked by β-antagonists was not preceded by a depression. Superfusion of dibutyryl cyclic AMP (10 µM - 1 mM) to the ganglia consistently and reversibly increased the non-cholinergic transmission in a manner similar to that produced by catecholamines. Lastly, intracellular iontophoresis of cyclic AMP also mimicked the enhancing effect of catecholamines on the non-cholinergic epsp. It is concluded that catecholamines with the exception of DA, exerted a biphasic effect on the peptidergic transmission of the inferior mesenteric ganglion cells: an initial depression that was mediated by α-adrenergic receptors and probably reflected a presynaptic inhibitory effect of catecholamines, whereas, the enduring facilitation was mediated by β-adrenergic receptors which appeared to be linked to activation of postganglionic cyclic AMP. (Supported by NIH grant NS15848 and USARMRDC-DAMD17-83-C-3133)

280.4 MIDBRAIN DA NEURONAL ACTIVITY IN MOUSE STRAINS WITH DIFFERING NUMBERS OF MIDBRAIN DA NEURONS. M.K. Sanghera and D.C. German, Depts of Psychiat. and Physiol., Univ. of Texas Health Science Center, Dallas, TX 75235.

Genetically inbred strains of mice have been found to possess differing numbers of midbrain dopamine (DA)-containing neurons. These differences have been correlated with spontaneous behavioral differences. For example, BALB/c mice have 20% more midbrain DA cells than CBA mice and, correspondingly they show greater spontaneous and DA drug-induced behaviors (Fink & Reis, Brain Res., 222:335-345, 1981). Examination of the tyrosine hydroxylase (TH)-labelled cells in these two mouse strains revealed that the differences in cell number occur within both the substantia nigra region and the ventral tegmental area (VTA) (German et al., Neurosci. Abs., 1150, 1983). In the nucleus accumbens (NAS) and caudate nucleus (CTA and substantia nigra target sites, respectively) we found that the CBA mouse had significantly higher apparent DA turnover than the BALB/c.

The purpose of the present experiment was to determine if these differences in apparent DA turnover rate could be explained by differences in the baseline firing rate of midbrain neurons. Extracellular single cell recordings were made from midbrain DA neurons in chloral hydrate anesthetized BALB/c and CBA mice. Recording sites were marked by the iontophoretic ejection of Fast Green dye from glass recording electrodes. The position of histologically verified recording sites were extrapolated onto TH-labelled DA cell density topography maps of BALB/c and CBA mice. A total of 55 single DA neurons were recorded. Cells in the substantia nigra zona compacta tended to fire faster in the CBA compared to the BALB/c (4.4 ± 0.6 vs. 3.0 ± 0.9 impulses/sec). In the VTA, however, there was no difference in firing rates (both groups 3.1 ± 0.4 impulses/sec). Thus, baseline firing rates of DA cells in the substantia nigra appear to be related to striatal DA turnover. The firing rates of VTA DA neurons, on the other hand, do not appear to be related to DA turnover in the NAS. However, since these DA neurons project to terminal areas other than the NAS, it is possible that their baseline firing rates are related to their projection sites. This possibility is presently under investigation. Research supported by grant MH-30546.

280.5 D_2-DOPAMINE RECEPTOR MEDIATES INHIBITION OF FORSKOLIN STIMULATED ADENYLATE CYCLASE ACTIVITY IN ANTERIOR PITUITARY. B. Borgundvaag* and S.R. George (SPON: P. Brawley). Dept. of Pharmacology, Univ. of Toronto, Toronto, Canada, M5S 1A8

It is well known that the inhibition of prolactin secretion by the anterior pituitary is controlled by the hypothalamic dopamine neurons. Further, it has been shown that this inhibitory effect of dopamine on prolactin secretion is mediated through the D_2 receptor. It has been postulated that the effect of dopamine on prolactin secretion is mediated via the coupling of the D_2 receptor to the enzyme adenylate cyclase (AC). Several groups have demonstrated the presence of a dopamine inhibited AC in anterior pituitary. While the inhibition of AC at concentrations of dopamine similar to those measured in hypophyseal portal plasma (4-20nM, Ben-Jonathan et al., Endocrinology 106:690, 1980), has been shown in cultured lactotrophs, this effect has hitherto not been demonstrated in tissue homogenates. We now report the inhibition of AC, in rat anterior pituitary homogenates, at physiological concentrations of dopamine. In addition, this is the first demonstration of dopamine inhibition of AC stimulated by the diterpine forskolin, in the anterior pituitary. AC was determined, in female rat anterior pituitary membranes, by measuring the conversion of ^{3}H-ATP to ^{3}H-cyclic AMP (cAMP), as described by Krishna et al., J. Pharmacol. Exp. Ther. 163:379, 1968. Forskolin stimulated AC activity in a dose-dependent manner. For studies examining inhibition, a concentration of 30uM forskolin, which produced a seventeen fold increase in cAMP production over basal levels, was used. The addition of dopamine inhibited forskolin stimulation of AC and this had a typically dopaminergic rank order of catecholamine potencies. Dopamine and dopaminergic agonists were shown to inhibit this forskolin stimulation by approximately 35% at maximal agonist concentrations. This result is consistent with the fact that forskolin stimulates AC in all cell types, while D_2 dopamine receptors are found primarily on lactotrophs, which in the female rat anterior pituitary, are known to comprise roughly 30% of the total cell population. The inhibition by dopamine of forskolin stimulated AC could be reversed in a dose-dependent fashion by the D_2 specific antagonist (+)-butaclamol, confirming that this effect was mediated through D_2 dopamine receptors.

280.6 PHARMACOLOGICAL DIFFERENTIATION OF DENTATE GRANULE CELLS AND HIPPOCAMPAL THETA NEURONS Rose, G.[1,2], Pang, K.[1], and Freedman, R.[1,2] (Sponsor: R.A. Harris) [1]Dept. of Pharmacology, UCHSC, and [2]Medical Research, VAMC, Denver, CO

Recordings from unanesthetized rats have allowed the identification of two classes of hippocampal neurons: complex-spike (CS) cells and theta cells. These two neuronal types differ from each other in both their electrophysiological characteristics and behavioral correlates. Because CS cells far outnumber theta neurons in area CA-1, it has been suggested that the former are the hippocampal pyramidal cells while the latter are interneurons. However, recent work (Rose et al., Brain Res., 266,29) has shown that, in the dentate gyrus, most granule cells also have the characteristics of theta neurons. We have recently demonstrated that locally applied phencyclidine (PCP), an indirect noradrenergic agonist, depresses the spontaneous firing of CA-1 CS cells but excites theta neurons recorded in the same region. In the present study we examined the effects of local application of PCP and norepinephrine (NE) upon neurons within the dentate gyrus.

Recordings were made from the hippocampal region of either urethane- or barbituate-anesthetized rats using 2-barrel glass micropipettes. The recording barrel was filled with 5 M NaCl, while the other barrel contained either 10^{-5} M PCP or 10^{-2} M NE in 165 mM NaCl. Drugs were applied using micropressure ejection. CS and theta neurons were identified by their characteristic unfiltered action potential duration. Stimulating electrodes were located in both the dorsal and ventral psalteria to activate entorhinal and commissural afferents to the hippocampus as an aid to verifying the location of the recording electrode.

Local application of PCP or NE onto CS cells in areas CA-1, CA-3 or the hilus caused depression of spontaneous discharge. In contrast, theta neurons in these regions were excited by either compound. Within the granule cell layer, however, 14 of 16 neurons with theta characteristics were depressed, while one CS cell was excited.

These results indicate that hippocampal neurons differ in the response to local application of NE or PCP. More, importantly, the agents evoke dissimilar responses from hippocampal theta neurons and dentate granule cells. Thus, two types of neurons which share common electrophysiological characteristics and behavioral correlates may be differentiated using a pharmacological criterion. Supported by USPHS grant DA02429 and the VA Medical Research Service.

280.7 NEURONAL DISCHARGE PATTERN: INFLUENCE ON A9 DA CELLULAR RESPONSE TO AUTORECEPTOR STIMULATION. P.D. Shepard and D.C. German, Depts. of Physiology and Psychiatry, Univ. of Texas Health Sci. Center, Dallas, Texas 75235.

Dopamine (DA)-containing neurons in the substantia nigra (SN) possess autoreceptors. Stimulation of these receptors by low doses of the DA agonist, apomorphine (APO), decreases DA cell impulse flow. Recent studies have demonstrated that the sensitivity of individual DA neurons to the rate-decreasing actions of APO is inversely related to the cell's spontaneous firing rate (Shepard & German, Neurosci Abs., 8:264.1, 1982; White & Wang, Life Sci., 34:1161, 1984). Thus, fast firing DA cells are generally less sensitive to the rate suppressant actions of APO than are slower firing DA cells. However, because DA neurons exhibit several different firing patterns, we sought to determine whether the cell's discharge pattern influenced the ability of APO to suppress neuronal firing rate. Extracellular, single unit activity of midbrain DA neurons was recorded in male rats anesthetized with chloral hydrate. Cells were tested with either a single dose or multiple doses of 5 µg/kg i.v. APO followed by a single dose of 0.1 mg/kg i.v. of the DA antagonist haloperidol. Cells were divided into two categories according to differences in pre-drug discharge pattern. Group 1 consisted of cells which exhibited tonic or "pacemaker-like" discharge patterns characterized by normally distributed interspike interval (ISI) histograms. Group 2 was comprised of cells which exhibited a phasic or "bursting" discharge pattern which gave positively skewed ISI histograms. Comparison of the sensitivity of cells within each group to the rate-decreasing effects of APO revealed that despite a significantly faster baseline firing rate (Group 1 = 3.7 ± 0.2 imp/sec; Group 2 = 5.85 ± 0.3 imp/sec; $t = 6.02$, $p < .001$) cells in Group 2 exhibited a significantly greater reduction in firing rate following the drug than cells in Group 1 (Group 1 = 0.64 ± 0.14 imp/sec; Group 2 = 1.81 0.25 imp/sec; $t = 4.04$, $p < .002$). These data are consistent with the hypothesis that cells which exhibit a phasic discharge pattern exist in a more depolarized state than cells which exhibit a tonic or pacemaker-like discharge pattern. It would therefore be expected that the extent of APO-induced hyperpolarization, and thus the magnitude of the reduction in firing rate produced by the drug, would be less for cells which initially exist in a relatively hyperpolarized state. Supported by grant MH-30546.

280.8 COMPARISON OF PRE- AND POSTSYNAPTIC DOPAMINE RECEPTORS IN THE MESOLIMBIC SYSTEM OF THE RAT BRAIN. F.J. White, M.M. Voigt and R.Y. Wang. Dept. of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

Considerable evidence suggests that, in the nigrostriatal dopamine (DA) system, presynaptic DA receptors (autoreceptors) are more sensitive than postsynaptic DA receptors. Although this phenomenon has also been reported for the mesolimbic system, there is little direct physiological evidence to support it. In the present experiments, we used single cell recording and microiontophoretic techniques to: (1) characterize the pharmacological properties of A10 DA autoreceptors in the rat ventral tegmental area and postsynaptic DA receptors in the nucleus accumbens (NAc), a forebrain limbic area which receives a dense DA innervation from A10 DA neurons and (2) compare the responsiveness of pre- and postsynaptic DA receptors to DA agonists. NAc neurons differed from A10 DA neurons in that only NAc neurons were inhibited by the D-1 specific DA agonist SKF-38393 (14 of 20 cells). Both NAc neurons and A10 DA neurons were suppressed by DA and the D-2 specific agonist LY141865 (LY). Some NAc neurons were inhibited by both LY and SKF. The D-2 specific antagonist sulpiride blocked the effects of LY on both NAc and A10 cells but failed to alter the inhibitory effects of SKF on NAc cells. When compared to NAc neurons, A10 DA neurons were at least 3-5 times more sensitive to the inhibitory effects of iontophoretic DA (0.01 M) and LY (0.01 M). Intravenous (i.v.) administration of LY caused a potent suppression of A10 DA unit activity (ED50=15 µg/kg). In contrast, the response of NAc cells to i.v. LY was usually biphasic such that low doses (7-63 µg/kg) increased the firing rate (by 30-70%) whereas higher doses decreased the firing rate (ED50=96 µg/kg). The initial increase was probably due to disinhibition consequent with A10 DA suppression. Thus, the D-2 DA autoreceptors on A10 DA neurons were at least 3-6 times more sensitive to DA agonists than the postsynaptic D-2 DA receptors on NAc neurons. Postsynaptic DA receptors in the olfactory tubercle were also less sensitive to LY than A10 DA cells. These results indicate that NAc neurons may possess both D-1 and D-2 DA receptors. In contrast, A10 DA neurons appear to possess only D-2 receptors which exhibit a higher affinity for DA agonists than postsynaptic D-2 receptors in limbic areas. (Supported by USPHS Grants MH-34424, MH-38794, MH-00378, MH-08886 and the Scottish Rite Schizophrenic Research Program N.M.J., U.S.A.)

280.9 COMPARISON OF DOPAMINE AGONISTS IN AUTORECEPTOR AND POST-JUNCTIONAL DOPAMINE RECEPTOR FUNCTION IN RAT STRIATUM. T.C. Westfall and L. Naes*. Dept. of Pharmacology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

There are numerous reports utilizing both in vivo and in vitro measurements that are consistent with the existence of autoreceptors on dopamine (DA) neurons in the central nervous system which modulate the release and/or synthesis of this neurotransmitter. It has also been observed that activation of postjunctional DA receptors results in a decrease in the evoked release of acetylcholine and glutamate. It is unclear if the receptors leading to these responses are pharmacologically similar or dissimilar. The purpose of the present study was to compare the relative potency of a series of DA agonists in altering DA synthesis (autoreceptor function) on the one hand, as well as inhibition of acetylcholine and glutamate release (postjunctional DA receptor function) on the other hand, as a means of addressing this question. Autoreceptor function was determined by measuring the inhibitory effect of DA agonists on the synthesis of DA (conversion of ^{3}H-tyrosine to ^{3}H-DA) in striatal slices. Postjunctional receptor function was determined by examining the inhibitory effect of similar drugs on the potassium evoked release of ^{3}H-choline or ^{3}H-glutamate from superfused slices initially incubated with ^{3}H-acetylcholine or ^{3}H-glutamate. The IC50's (μM) for apomorphine, epinine, piribedil, lergotrile and bromocriptine in inhibiting DA synthesis were 0.70, 2.3, 8.0, 22 and 5 μM. The IC50's of this same series of drugs in decreasing the K$^+$-induced release of ^{3}H-choline were 1.5, 2.0, 0.86, 3.3 and 3.5 μM while for inhibiting ^{3}H-glutamate release they were 0.5, 0.5, 0.28, 0.11 and 0.06. The rank order of potency of the various agonists in reducing DA synthesis was apomorphine>epinine>DA>piribedil>lergotrile>bromocriptine. The rank order of potency in inhibiting ^{3}H-choline release was bromocriptine>ADTN=piribedil>apomorphine>epinine>lergotrile while the rank order of potency in inhibiting ^{3}H-glutamate release was bromocriptine>lergotrile>piribedil>apomorphine=epinine>ADTN. These results demonstrate that there are differences in the potency and rank order of potency for various DA agonists in modulating DA synthesis compared to altering acetylcholine or glutamate release. It is suggested that the receptors involved in mediating these various responses are pharmacologically different. (Supported in part by NIH-NS 16215.)

280.10 AMPHETAMINE ACTION ON TERMINAL EXCITABILITY AND IMPULSE TRAFFIC IN NORADRENERGIC LOCUS COERULEUS NEURONS. L.J. Ryan*, J.M.Tepper, S.J.Young* and P.M. Groves. Dept. Psychiatry, Univ. Calif. San Diego, La Jolla, CA 92093.

Activation of autoreceptors on locus coeruleus terminals in frontal cortex by noradrenergic agonists such as clonidine reduces the electrical excitability of the preterminal axon. Excitability is monitored by measuring the current required to elicit antidromic responses (Nakamura et al., 1982, Neurosci. 7:2217-2224). Conversely, noradrenergic antagonists increase terminal excitability. Systemic low dose amphetamine (AMP) acts like an antagonist, increasing terminal excitability whereas local infusion of AMP into the terminal field acts like an agonist, reducing excitability.

The effects of various doses of intravenously administered AMP on terminal excitability, firing rate, and antidromic action potential invasion of the soma and dendrites were studied in noradrenergic neurons of the locus coeruleus in urethane anesthetized rats. Low dose AMP (0.25 mg/kg) decreased threshold current (-9.68% ± 1.1%, n=60) while reducing firing rate from 1.59 sp/s (±0.16) to 0.91 sp/s (±0.11). No change in the likelihood of somatodendritic antidromic invasion was seen. Subsequent injection of higher doses of AMP further reduced firing rate (1.0 mg/kg: 0.16 sp/s ± 0.08, n=10; 2.5 mg/kg: 0.07 ± 0.03, n=6; 5.0 mg/kg: 0.04 ± 0.02, n=17) and caused failure of antidromic somatodendritic invasion. The two intermediate doses (1.0 and 2.5 mg/kg) had no further effect on threshold current (1.0 mg/kg: -1.41% ± 2.48; 2.5 mg/kg: -2.98% ± 3.10) whereas the highest dose (5.0 mg/kg) consistently reversed the effects of 0.25 mg/kg, increasing threshold current for antidromic activation (+9.43% ± 1.65). Both the increase in threshold current and the decrease in antidromic somatodendritic invasion caused by 5.0 mg/kg AMP, i.v., were reversed by 0.5 mg/kg yohimbine (threshold: -9.76% ± 1.36, n=7) without reinstating neuronal firing (0.10 sp/s ± 0.10).

These results suggest that at low doses AMP facilitates neurotransmission within the locus coeruleus, inhibiting neuronal firing. This, in turn, reduces transmission in the frontal cortical terminal fields, reducing pre- and postsynaptic receptor activation and so increasing terminal excitability. At a sufficiently high dose, AMP decreases terminal excitability despite the absence of impulse traffic. Since yohimbine blocks this effect on terminal excitability, high dose systemic AMP is acting indirectly as an agonist for pre- and postsynaptic alpha$_2$ receptors.

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280.11 EFFECTS OF ALPRAZOLAM, A NOVEL TRIAZOLO-BENZODIAZEPINE, ON LOCUS COERULEUS UNIT ACTIVITY. S.J. Grant, R. Mayor*, and D.E. Redmond, Jr. Primate Research Facilities, Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06510.

Alprazolam is a benzodiazepine derivative with a novel triazolobenzodiazepine structure and a wide spectrum of psychoactive properties. Although diazepam and other classical benzodiazepines are not considered effective in the treatment of depression or panic attacks, alprazolam has been shown to have significant clinical antidepressant and anti-panic activity, as well as anxiolytic properties. Both depression and panic attacks are commonly responsive to treatment with agents which alter noradrenergic function. In addition, diazepam and other traditional benzodiazepine agonists decrease noradrenergic unit activity. Since alprazolam has clinical effects similar to psychoactive agents known to decrease noradrenergic unit activity, it was hypothesized that alprazolam would also decrease noradrenergic unit activity.

Consistent with its greater clinical potency, alprazolam significantly reduced single unit activity in the locus coeruleus of anesthetized rodents by 50% at a dose of 0.02 - 0.06 mg/kg (i.v.), compared with the 0.1 - 1.0 mg/kg (i.v.) dose range previously reported for diazepam. Like diazepam, larger doses of alprazolam did not produce significantly greater decreases in unit activity. In addition, the specific benzodiazepine antagonist, RO 15-1788, blocked the effects of alprazolam.

These data demonstrate the potent effects of alprazolam on noradrenergic unit activity, an effect consistent with the action of most other anti-anxiety and anti-panic compounds. Preliminary evidence indicates that the effects of alprazolam on noradrenergic activity may be mediated through benzodiazepine receptors. However, additional studies are required before a direct action by alprazolam on adrenergic or other receptors can be ruled out.
(Supported by MH 31176, MH 25642).

280.12 EXPERIMENTAL STROKE ALTERS SPONTANEOUS AND SENSORY-EVOKED DISCHARGE OF LOCUS COERULEUS NEURONS. S. Aston-Jones*, G. Aston-Jones and M. Ennis* (SPON: S. L. Foote). Center for Neurobehavioral Sciences, SUNY, Binghamton, NY 13901; present address: Dept. Biology, NYU, New York, NY 10003.

Previous studies report decreased norepinephrine (NE) levels in cerebrocortex and locus coeruleus (LC) following experimental stroke in rat. These findings, in light of emotional changes (e.g., depression) that often follow stroke in humans, led us to investigate the effects of middle cerebral artery (MCA) occlusion on discharge characteristics of NE-containing LC neurons.

The right MCA was carefully occluded by ligation under microscopic control in 11 Sprague-Dawley adult male rats. Individual LC neurons were recorded in chloral hydrate anesthetized subjects, either 24 h (N=6 rats) or 5 d (N=5 rats) following experimental stroke. Similar recordings from 7 intact rats served as control data. All recordings were histologically verified as being from NE-LC neurons.

Spontaneous discharge of LC neurons 24 h post-stroke was significantly higher (2.7 ± 0.3 Hz; N=39 cells) than in control subjects (1.5 ± 0.2 Hz; N=24 cells; $p<.01$). In animals recorded 5 d after stroke, 6 of 36 LC neurons were nonspontaneous (compared to 0 of 24 cells in control animals), and the remaining 30 cells had an average rate of 1.2 ± 0.1 Hz. Sensory responsiveness of LC neurons was tested using subcutaneous electrical stimulation of the contralateral rear foot. Response magnitudes were significantly increased at 24 h, but not 5 d, following stroke. This increased response consisted of an enhanced "late" excitation, occurring 100-300 msec after foot shock. In contrast, during this same response interval in animals 5 d post-stroke, activity was significantly lower than in control subjects ($p<.01$). The magnitudes of the initial excitatory response (15-50 msec post-stimulation) were similar for all 3 groups of animals. These results indicate that the characteristic period of inhibition following activation of LC neurons may be transiently suppressed, and then enhanced, at various times following stroke.

Our findings indicate that ischemic insult may have global brain consequences, perhaps in part by altering physiologic properties of diffusely projecting noradrenergic LC neurons.

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281.1 REDUCTION IN CHOLINE UPTAKE IN RAT MODELS OF LEARNING DISABILITIES. B. Frieder and V.E. Grimm* Dept. of Isotope Research , The Weizmann Institute of Science , Rehovot, Israel,76100.

Some forms of learning disabilities can be caused by traumatic events or chemical insults during gestation or birth. Diazepam (DZP),20mg/kg p.o. given to rat mothers during the last two weeks of pregnancy resulted in a learning deficit. The deficit was observed only in a complex brightness discrimination maze where the rewarded stimulus and the non-rewarded stimulus were presented simultaneously at six choice points,but not in a successive brightness discrimination task where the animal was presented only one stimulus on any given trial. Perinatal anoxia (pure N_2 for 25 minutes within 24 hours after birth) and postnatal DZP (during the first two weeks of lactation) resulted in a similar learning deficit. DZP exposure during the last week of gestation seems to be sufficient for causing learning disabilities,while exposure to DZP during the second week of gestation did not affect learning.

Uptake of choline to the synaptosomal fraction in the frontal cortex was significantly reduced in the prenatally treated male adult offspring by 25% , in the postnatally treated adult offspring by 38% and in the anoxia treated offspring by 16% . Norepinephrine and GABA uptake remained intact. The learning deficit was more pronounced in the male offspring. The female offspring exposed to DZP prenatally or to anoxia perinatally did not show a significant learning deficit and their choline uptake in the cortex was not affected. Females exposed to DZP during lactation showed some learning deficits, but in this case choline uptake was not changed. Uptake of serotonin to the synaptosomal fraction in the frontal cortex decreased and GABA uptake in the hippocampus was increased. Reduction in choline uptake in the frontal cortex might be a common mechanism in some forms of learning disabilities.

281.2 CARDIOVASCULAR EFFECTS OF INTRAVENTRICULAR HEMORRHAGE IN NEONATAL SWINE. P.M. Gootman, H.L. Cohen, N. Gootman*, P.S. Griswold*, B.J. Buckley*, E. Weinhouse*. Dept. Physiol. Downstate Med. Ctr., SUNY, Brooklyn, N.Y. 11203 and Div. Ped. Cardiol. Schneider Children's Hospital, LIJ-HMS, SUNY, Stony Brook, New Hyde Park, N.Y. 11042.

An acute increase in intracranial pressure is known to result in an elevation in arterial pressure accompanied by a decrease in heart rate (Cushing, Am. J. Med. Sci. 1902, 124: 375). Since intraventricular hemorrhage (IVH) is the most common serious neurologic event of the neonatal period (Tarby & Volpe, Ped. Clin. N.A., 1982, 29:1077), we decided to examine the cardiovascular (CV) changes occurring to experimentally induced IVH. Piglets $<$ 24 hours old were studied using our standard methodology (Gootman, et al., Fed. Proc. 1983, 42:1648). Animals were placed in a stereotaxic apparatus and needles inserted into the right lateral ventricle (LV) (verified histologically) and into the lumbar spinal canal (for measurement of cerebrospinal fluid pressure, CSFP). Aortic pressure (AoP), carotid (Car), femoral (Fem) and renal arterial blood flows were recorded simultaneously with EKG (HR) and end-tidal CO_2. IVH was simulated by sequential injection of 0.5 ml of the animal's own blood into the LV. In a control group, artificial cerebrospinal fluid (ACSF) was injected. IVH was induced or ACSF injected, at 10 min intervals, to a total of 8-10 ml blood or ACSF. Following the second injection into LV, blood appeared in the spinal column, CSFP increased with increasing total volume of blood injected. Each injection increased CSFP 10-20 cm H_2O. Control group CSFP did not significantly change. Significant immediate increase in $\overline{AoP}$ was obtained at total of 2.5 ml IVH. This threshold for a pressor response corresponded to a peak increase in $\overline{CSFP}$ of 39.2±6.8 cm H_2O. The magnitude of the increase in $\overline{AoP}$ was greater as the total ml IVH increased. Significant CV responses: Stage 1 IVH (4 ml): increased $\overline{AoP}$, decreased HR, Car and Fem vasoconstriction. Stage 2 IVH (8 ml): decreased AoP, and increased HR. In neonates, IVH produced an initial Cushing response with CV collapse occurring as IVH progressed ($>$ 5 ml). (Supported by NIH grant HL-20864)

281.3 ABNORMALITIES OF CORTICAL DEVELOPMENT IN MURINE TRISOMY 16, AN ANIMAL MODEL FOR DOWN'S SYNDROME. M. E. Blue, M. E. Molliver, J. D. Gearhart* & J. T. Coyle The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Trisomy 16 in the mouse is homologous to human trisomy 21 (Down's Syndrome) in terms of synteny of gene loci and several common phenotypic features; it may serve as an animal model for Down's Syndrome (D.S.). We have examined fetal mice with Ts-16 on days E13-E18 to determine whether there are structural abnormalities in the cerebral hemisphere. Preliminary findings indicate that the mouse with trisomy 16 exhibits substantial alterations in the proliferative compartments of the forebrain and in early development of the cortical plate. 1) There is a reduction in the overall size of the telencephalic vesicle and a corresponding decrease in the surface area of the pallium. 2) The cortical plate does not extend as far medially as in controls indicating a decrease in the tangential growth of the cortex. 3) In the radial dimension, there is a decrease in overall thickness of the pallium and its constituent zones, particularly the cortical plate, sub-plate, and subventricular zone. The preceding findings lead to the proposition that abnormal brain development in Ts-16 results from a decrease in cell proliferation in the ventricular zone, which is likely to produce an impairment of tangential and radial growth of the pallium. As a consequence, the cortical surface area should be substantially decreased and there might be a failure to produce the full complement of cortical neurons. The decreased cortical surface in the Ts-16 mouse is consistent with related findings in human D.S.: low brain weight, decreased size of the hemisphere and abnormal cortical convolutions, namely, shallow primary sulci, and a paucity of secondary sulci. Since the hemisphere is highly convoluted in man and over 2/3 of the cortical surface lies within sulci, the decrease in sulcation is evidence for a decrease in cortical surface area. Based on the present findings, the decrease is likely to result from impaired tangential growth of the hemisphere secondary to decreased neuroblast proliferation in the embryo. We postulate that a decrease in tangential growth may lead to the formation of a diminished number of cortical columns and that reduced cell proliferation may produce a decreased number of neurons in each column. Therefore, the mental deficit in Down's Syndrome may be due to a decrease in the number of information processing modules and possibly a decreased number of cells within each module.

281.4 NEUROEPITHELIAL-MESENCHYMAL RELATIONS IN THE MORPHOGENESIS OF EYE ABNORMALITIES IN A MUTANT AND RADIATION INDUCED AQUEDUCT STENOSIS-HYDROCEPHALUS SYNDROME. R. A. Glover*, C. J. D'Amato and S. P. Hicks. Depts. of Anatomy and Cell Biology, and Pathology, University of Michigan Medical Center, Ann Arbor, Michigan 48109.

In a recessive mutation in the rat, discontinuities of the basal lamina (BL) were observed between the neuroepithelium and mesenchyme of the midbrain-thalamic junction (MTJ) around the 11th fetal day. These discontinuities were accompanied by premature contacts between these two cell populations, neural ectopias were observed protruding into the mesenchyme and there was a thickening of the MTJ walls leading to prenatal stenosis of the cerebral aqueduct with hydrocephalus late in gestation (Glover, D'Amato, Hicks, Neurol. 33, 221, 1983). A similar sequence of events was seen in normal fetuses subjected to radiation with 225R on the 11th fetal day (Glover, D'Amato, Hicks, Neurosci. Abstr. 9, 595, 1983).

In both of these groups of animals a variety of eye abnormalities were commonly expressed. Best studied in the mutant, these abnormalities ranged from a distorted little eye close to where the optic nerve enters the cranium, rudiments of retina strung along the course usually taken by the optic nerve, or a full-size eye with a retina that had not invaginated so that the globe was all retina with the ganglion cell layer on the outside. In some full-size eyes where the retina invaginated forming a "normal" structure with ganglion cell layer inside, the posterior part of the eye also formed a retina instead of a pigment layer and associated structures, and the ganglion layer was on the outside. The two retinas apposed each other as mirror images.

Electron microscopy of the optic cup in the mutant fetuses about the 11th fetal day has shown morphological alterations similar to those seen in the MTJ region. Discontinuities were observed in the BL accompanied by altered neuroepithelium-mesenchyme relationships. Also preliminary studies show that these same altered features are seen following 225R on the 11the fetal day. The mutants survive to reproduce, the 225R rats die at birth. Occasional hydrocephalics with eye defects follow 170R on the 11th fetal day with postnatal survival. Fetuses exposed to 150R show only mild eye defects.

281.5 PREMATURE HUMAN INFANTS WITH AND WITHOUT INTRAVENTRICULAR HEMORRHAGE: ANALYSIS OF MOVEMENT. C.R. Almli and A.L. Lawler*. Dept. Prevent. Med., Prog. Occupat. Ther., Washington University School of Medicine, St. Louis, MO. 63110.

Little is known about the motor movements of premature human infants in spite of the fact that movement is a major component of the premature infants' behavioral repertoire. Further, premature infants display a high incidence (40% to 60%) of neuropathology. The literature on movement of premature infants is typically anecdotal, general and often inconsistent. The present study describes the movements of premature infants, and compares the movements of infants sustaining intraventricular hemorrhage (IVH) with infants without IVH (NON-IVH).

Premature infants (28 to 32 weeks EGA) of the NON-IVH and IVH groups were filmed (25 frames per sec) while supine at 6 to 10 days postnatal. Infants heads were positioned on the midline. IVH was diagnosed by repeated CAT scans.

The films were analyzed frame by frame for movements of the arms and legs. The NON-IVH infants were found to be moving their arms and/or legs 26% of the time, and they displayed a movement burst each 21.4 sec. Movement burst duration of the NON-IVH infants was 5.6 sec. In contrast, the IVH infants displayed less movement (20% of time active), a higher frequency of movement bursts (each 12.3 sec) and a shorter movement burst duration (2.5 sec). The NON-IVH infants displayed a higher frequency of right limb than left limb movements, while this was reversed for the IVH group.

The NON-IVH and IVH infants were similar in that both displayed a higher incidence of forearm than upper arm movements, while the frequency of thigh and lower leg movements were equal. Also, both groups were similar in displaying a higher frequency of arm than leg movements.

These results indicate that premature infants tend to display relatively stereotyped movement patterns, i.e., plane movements of less than 15 degrees excursion in the pro-or iso-gravity mode. However, infants sustaining known neuropathology (IVH) may be discriminated from other premature infants on the basis of a number of movement variables including total number of movements, movement burst duration and movement laterality. (Supported by Grant USHRSA-51998)

281.6 NEONATAL 6-HYDROXYDOPAMINE TREATMENT AND AGING ALTER THE APOMORPHINE DISCRIMINATIVE "CUE." J.T. Concannon and M.D. Schechter. Program in Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Five-day-old pups were administered desmethylimipramine intraperitoneally prior to intracisternal administration of either 6-hydroxydopamine or its vehicle. Six-hydroxydopamine-treated rats were hyperactive in the open-field at 30 days of age, relative to controls, and were depleted of whole-brain dopamine (27.2% of controls). The remaining animals in the litters were trained to discriminate the interoceptive cue produced by intraperitoneal apomorphine (0.16 mg/kg as base), in a 2-lever, food-motivated operant task, starting at approximately 35 days of age.

Learning curves for the 6-hydroxydopamine-treated and control groups were similar over 80 training sessions. Mean ($\pm$ SEM) sessions to criterion performance were also similar (33.4 ± 6.5, for 6-hydroxydopamine; and 35.0 ± 6.5 sessions for controls) indicating that neonatal 6-hydroxydopamine treatment had little effect on learning rates. Littermate 6-hydroxydopamine-treated animals, sacrificed when the trained group reached criterion, were depleted of whole-brain dopamine (30.8% of controls). Despite similar learning rates, the 6-hydroxydopamine-treated rats were hypersensitive to various doses of apomorphine (0.04-0.24 mg/kg) tested in extinction. Furthermore, the dose-response curves for the 6-hydroxydopamine-treated and control groups were parallel. In a separate experiment, aged (25-month-old) rats were similarly trained to discriminate between 0.16 mg/kg apomorphine and saline. Dose-response determinations with various doses of apomorphine (0.04-0.24 mg/kg) indicated responding equivalant to the rats treated neonatally with 6-hydroxydopamine.

These results suggest that the 6-hydroxydopamine-treated and aged rats are behaviorally supersensitive to a direct-acting dopamine agonist. This supersensitivity may be mediated by an increased number of dopamine receptors produced by the neonatal 6-hydroxydopamine treatment or by alteration in dopamine function accompanying aging. (Supported by NIMH grant #33636)

281.7 PHYSIOLOGY AND DEVELOPMENT OF THE VISUAL SYSTEM IN CATS WITH CONGENITAL MICROSTRABISMIC ESOTROPIA. A. Schoppmann, R. Nikel*, K.-P. Hoffmann. Abt. für Vergleichende Neurobiologie, Universität Ulm, D-7900 Ulm, FRG.

We have studied congenital misalignment of the eyes in members of a cat colony inbred from parents who displayed abnormal esotropia. Among twenty-seven offspring, 15 had their visual axes crossed between 2.1 and 7° outside the normal variability under anesthesia and paralysis. Visual alignment was measured as the crossover of receptive fields of binocular cells in area 17. The alignment in alert state was indirectly determined through correction of the horizontal eye movements caused by relaxation of the extraocular eye muscles following curarization. This correction increased the convergence by an average 5.5°. In the twelve less prominent cases visual disparity values lay inside the range of variation of 12 control cats not related to the inbred colony, but still mostly on the side convergent to the controls' average disparity (2.6° uncrossed).

Binocularity was disrupted in area 17 of the inbred cats with the strongest phenotypes showing between 50 and 70 % monocular cells (controls 18 % on the average). We observed a correlation between visual disparity and ocular dominance in that the strongest esotropes had the least binocular excitatory convergence. We saw a graded loss with increased esotropia and no sign of monocular suppression as would be found in strabismic amblyopia. The most prominent cases were rather characterized by equal influence of the two eyes (U-shaped ocular dominance diagrams).

In behavior, the performance in a depth perception test was not better in the binocular viewing situation, than with one eye occluded. In contrast, control cats showed an improved performance with both eyes open.

Pupillographic measurements in the alert state revealed a coincidence of abnormal divergence of the optic axes and of a convergence of the visual axes in kittens from eye opening throughout the first half year of life. Pupil divergence in behavior was found the more pronounced, the more the visual axes were crossed with the eye muscles relaxed.

Retinal measurements make it likely that an anatomical anomaly might be the primary defect causing the misalignment. There is evidence that the area centralis is temporally displaced with respect to the axis of symmetry of the eye, thus producing the crossover of the visual axes in paralysis, and explaning pupil divergence as a compensatory effort.

281.8 UNRESPONSIVE, A TRANSIENT MUSCLE DYSFUNCTION MUTANT IN XENOPUS LAEVIS. R. Tompkins, F. E. Dudek, C. F. Ide and J. Fuseler*. Dept. Biology, Tulane Univ., New Orleans, LA 70118 and Dept. Physiology, Tulane Univ. Med. School, New Orleans, LA 70112.

Xenopus laevis embryos homozygous for the recessive mutant unresponsive fail to move until just prior to feeding stage, after which they recover slowly. Previous grafting analysis showed that both the motoneurons and the skeletal muscles of mutant embryos were affected, but that each could function normally in association with the other tissue of normal genotype.

The electrophysiological properties of the muscle fibers of mutant embryos were evaluated qualitatively with intracellular recording. The resting potentials (-60 to -90 mV) and spontaneous end-plate potentials (EPP's, up to 80 mV) appeared normal. In particular, characteristic bursts of large spontaneous EPP's were observed. In addition, extracellular nerve stimulation or a decrease in illumination evoked similar bursts of EPP's. Occasionally, action potentials were observed on the peaks of EPP's. These data indicate that the lack of movement of mutant embryos is probably not due to a genetic defect in the electrical properties of either the nerve or muscle.

Electrophoresis showed that all major muscle proteins are present in mutant embryos in normal concentrations. Histology revealed myofibrillar disorganization. Mutant myofibrils were not alligned in parallel arrays as in normal muscle cells. Rather, they appeared twisted and, occasionally, braided. The myofibrillar bundles, viewed in polarizing light, appeared to vary in thickness along their lengths. Calcium-induced chlortetracycline fluorescence was greatly reduced in mutant muscle cells, suggesting that a lack of intracellular membrane-bound calcium is the immediate cause of the failure of mutant embryos to move.

These data, when combined with previous grafting data, further suggest that motoneurons and muscles both normally produce a factor(s) necessary for normal skeletal muscle calcium metabolism during early development, and that mutant motoneurons and skeletal muscles are deficient in this regard.

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281.9 WEAVER MUTANT GENE EXPRESSION IN CULTURES OF NEONATAL MOUSE CEREBELLUM. P.A. Johns* and M. Willinger* (SPON: L. Siegel). Depts of Neuroscience, Children's Hospital, Neuropathology, Harvard Medical School, Boston, MA 02115.

The weaver mutation results in a dose-dependent impairment of cerebellar external granule cell viability and translocation. To determine if the weaver gene is expressed in the cerebellum prior to the onset of granule cell generation and translocation at P4, we have quantitiated small neuron viability in dissociated cerebellar cultures of newborn (P0), P4 and P7 mice carrying the weaver mutation. Since weaver mice cannot be identified phenotypically prior to P4, wv/wv dams were bred with +/wv males and cerebella of the progeny were cultured individually. Properties reflecting expression at the weaver locus should segregate according to the expected 1:1 ratio of wv/wv and +/wv progeny. Cerebella of individuals from P0, P4 and P7 mutant litters and from age-matched normals were dissociated into single cells and cultured as monolayers. At days 1 and 4 in vitro, the cultures were fixed and those neurons 7-9 um in diameter, displaying high nuclear/cytoplasmic ratios were counted. In normal controls at day 1 in vitro, these cells represent 47, 83 and 83 percent of surviving P0, P4 and P7 neurons respectively. By day 4, 60, 93 and 83 percent of the small neurons present at day 1 still remain. In cultures of mutant individuals, at each postnatal age surveyed, small neuron viability by four days in vitro segregated 1:1 into two distinct groups. In cultures of cerebella from P0 mutant litters, the percentage of small neuron survival in the two classes were 14+3 and 37+7 for one litter; 33+1 and 50+5 for another litter. In P4 mutant cultures the percent of small neurons remaining in the two groups was 18 and 32 percent; for P7, 23 and 57 percent . Neurite lengths of surviving neurons were decreased in P4 and P7 mutant cultures but not in P0 mutant cultures. Preliminary analysis of the time course of cell loss in mutant cultures of all age groups indicates that the loss occurs throughout the four days in culture. In conclusion, the presence of the weaver gene results in a dose-dependent decrease of small neuron viability in cultures of neonatal cerebellum. These results suggest that the gene may be expressed prior to and independent of granule cell translocation and cerebellar lamination. Supported by NS17225, MOD-781 (MW) and HD18655-02.

281.10 IDENTIFICATION OF RISK INDICATORS FOR THE SUDDEN INFANT DEATH SYNDROME (SIDS) UTILIZING DISCRIMINANT ANALYSIS OF SLEEP-WAKING PHYSIOLOGICAL DATA. Z. Frostig* and R. M. Harper. Dept. of Anatomy and Brain Research Institute, UCLA, Los Angeles, CA 90024.

We are assessing the development of physiological parameters to find risk indicators for SIDS. Twelve hour all-night recordings of physiological data were obtained at 1 wk and at 1, 2, 3, 4, and 6 months of age from a group of 25 control infants and a group of 25 siblings of SIDS victims. Minute-by-minute measures of eye movements and muscle activity, filtered and integrated EEG at delta, theta and sigma bands, and cardiac and respiratory rate and variability were calculated, and each 12 hr set of values was subjected to spectral analysis. Coherence measurements from 0.23 to 21.0 cycles/hr were determined between parameters. Statistical procedures were oriented towards reducing the large number of variables to a minimum required for maximizing the difference between risk and control groups. Reduction of the number of variables was done in 3 steps: 1) A comparison of group means and group trends with age on each of the physiological measures, utilizing a two-way mixed model ANOVA; 2) Evaluation of group means or an age-trend index of the physiological measures over the 6 months being studied; and 3) Step-wise discriminant analysis using the percentage of correctly reclassified subjects as a criterion for limiting the number of variables entering the classification function. The final classification function included only 3 variables. This function correctly reclassified 96% of the subjects to their respective groups. All 3 variables were coherence measures which included respiratory rate (RR): 1) RR/delta EEG at 12 c/hr; 2) RR/median heart rate at 15 c/hr; and 3) RR/delta EEG at 0.5 c/hr. Developmental trend plots indicated that high frequency variables (12 c/hr RRMD/delta EEG, and 15 c/hr RRMD/HRMD) in the risk group are accelerated with respect to controls, while the low frequency variable (0.5 c/hr RRMD/delta EEG) developed later in risk infants than in controls. Since variables included in the final classification function are not correlated, a subject from the risk group may have one or more of the abnormalities mentioned above. These findings indicate that the development of temporal coupling of physiological variables may be a useful index in describing SIDS risk parameters. Supported by HD 14608-03.

281.11 THE EFFECTS OF PHENOBARBITAL ON THE DISTRIBUTION OF SEROTONIN FIBERS IN THE RAT CEREBELLAR CORTEX. R.S. Hannah*, A.W. Spira and S.H. Roth. Depts. of Anatomy and Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta, Canada. T2N 4N1

The pattern of distribution of serotonin (5-HT) immunoreactive nerve fibers was studied in the rat cerebellar cortex to determine the long term effects of perinatal phenobarbital (PB) administration. Perinatal PB administration has been demonstrated to reduce Purkinje cell numbers, (Hannah et al., Teratology, 1982, 26:21). Time pregnant Long Evans hooded rats were administered either PB (10 mg/kg) or normal saline beginning on day 18 postcoitus, on a once-daily basis until day 21 postnatal. The pups were maintained until they were sacrificed at six months of age.

The animals were perfused transcardially with 4% paraformaldehyde in phosphate buffered saline. The cerebella were removed and sectioned both in the frontal and sagital planes with a freezing microtome (30-50 µm thick). Immunofluorescent staining was carried out according to the method of Hokfelt, utilizing commercial 5-HT antibody (Immuno Nuclear). The study was restricted to the vermal region. In general, the 5-HT immunoreactive nerve fibers in the control animals were similar to the three types reported by Takeuchi et al., (Cell and Tiss. Res., 1982, 226:1). The majority of immunoreactive fibers were found in the molecular layer, especially in frontal sections. The most predominate fiber resembled the parallel fiber-like elements reported by Chan-Palay (Anat. Embryol., 1975, 148:235) and tended to be most numerous in the superficial areas of the molecular layer. No mossy fiber rosettes were observed in either control or treated groups.

The treated group differed from the control group in two areas. In the molecular layer, in frontal section, the parallel-like fibers were more evenly distributed with the highest number in closer proximity to the Purkinje cell layer. Also in the molecular layer, in sagital section, numerous fibers were observed, running directly on top of the Purkinje cell layer. This arrangement was not observed in any of the control animals. The results indicate that PB treatment produces an architectural alteration in 5-HT nerve fibers. Whether or not the observed alterations are the result of a direct or indirect drug effect, it is likely that the abnormal 5-HT fiber input could result in functional alteration.

(Supported by the Alberta Mental Health Foundation).

281.12 NEUROBEHAVIORAL DEVELOPMENT IN THE TWITCHER MOUSE AND HETEROZYGOUS LITTERMATES. Ch. E. Olmstead, UCLA/MRRC Res. Grp., Lanterman State Hospital, Pomona CA 91769.

The mutant twitcher mouse (C57BI/6J-twi), first described by Duchen et.al. (Brain 106, 1980) is an enzymatically authentic (Kobayashi, et.al., Biochem. Med. 27, 1982) model of the recessively transmitted Globoid cell (Krabbe's) leukodystrophy. The specific enzyme deficit is galactosylceramidase and affected and carrier individuals can be determined by enzyme assay done on clipped tail. The studies on neurobehavioral development reported here were carried out to develop a better understanding of the functional pathophysiology of this demyelinating disease in both the homozygously affected and heterozygous carriers.

Enzyme assays. Between 10 and 15 days of age, 0.5cm lengths of tail were clipped and galactosylceramidase was determined by an assay adapted from Suzuki (Meth. Enzymol. 50, 1978). Affected homozygous, heterozygous carriers and normal animals were readily identified and were assigned to groups based on sex and genotype.

Neurological Tests. Data will be reported from a complete neurological battery with emphasis placed on the development of the grasp reflex and the contact placing response (CPR). CPR. There were significant developmental changes in both carrier and affected animals. Normal animals were fully developed compared to heterozygote and affected by day 15. Significant differences between groups were clearly apparent only after day 20 and were due to the regression of the CPR in the affected animals. Grasp. Significant developmental effects were seen in both heterozygote and normal animals, but the affected animals never showed an adequate response. There were significant differences between groups from day 15 onward with the affected animals consistently the worst.

Neurobehavior. Rotorod. Significant developmental effects in the ability to remain on a 4cm rotating dowel were seen in all groups. Homozygous females were affected earlier than males and there were also significant differences between heterozygous males and females. Hangtime was the time the mouse could hang from an inverted piece of 3/8" hardware cloth. On this task, there were significant differences between normals, heterozygotes and affected. Within each group, males were clearly superior.

In addition to documenting the progress of the disease in the affected animals, these data suggest 1) that females are at affected earlier than males and 2) female carriers might be at risk if one were to manipulate stress-inducing factors e.g. diet, temperature, or rearing conditions.

281.13 AUTISM AND EXTREMITY ASYMMETRIES. H.V. Soper, P. Satz*, and D.L. Orsini. Department of Neuropsychology, UCLA-NPI, and UCLA-NPI Research Program, Box 'A', Camarillo, CA 93011.

The autistic show a high rate of non-right-handedness, strongly suggesting cerebral pathology (Soper & Satz, in press). By our model, about 83% of the sinistrals would have damage predominantly on the left, and the converse would be true for about 73% of the dextrals. In general, early trauma to the postcentral cortex has been associated with reduced growth of the contralateral extremities (e.g., Silverstein, Neurology, 5: 30, 1955), and epileptics with early onset unilateral foci show such asymmetries (Satz et al., in press). The autistic have been reported to show minor physical anomalies (Steig & Rapoport, J. Aut. Child. Schiz., 5: 229, 1975). Hence, it was thought that the autistic would show substantial asymmetry in the length of the extremities in general, and the different handedness subgroups (right - 40%, ambiguous - 40%, left - 20%) would show different asymmetry distributions.

Manual. The length of each hand of 47 low functioning autistics (DSM-III diagnosis) was measured twice, and a mean difference (right minus left) score was computed. These scores were correlated with handedness score (6 trials on each of 8 tasks), and the scores compared for the different handedness groups. The correlation (-.14) was insignificant and the subgroup means and distributions were very similar.

Pedal. The length of each foot of 46 of the subjects described above was measured twice, and the same comparisons were made as for the hand measures. As with the hand measures, there was an insignificant correlation with handedness (+.025) and no difference between handedness groups. Also, the hand and foot measures were not related ($r = -.18$).

This lack of a relationship between extremity asymmetries and handedness was unexpected in view of the substantial indications of CNS pathology based on the handedness distribution. However, it is consistent with the general lack of primary sensory dysfunction found among the autistic (e.g., see Damasio & Maurer, Arch. Neurol., 37: 504, 1978). Taken together, the handedness and anthropometric data suggest a possible disruption of secondary and tertiary areas but not primary areas within the autistic.

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281.14 THERAPY ATTENUATES THE EFFECTS OF NEONATAL CORTICAL LESIONS IN ANIMALS GIVEN THE EARLIEST LESIONS. B. Kolb and I. Q. Whishaw, Dept. of Psychology, University of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

We have found that removal of the frontal cortex of rats in infancy fails to allow significant sparing of function on tests of simple motor and more complex species-typical behaviors and allows only partial sparing on tests of learning. Furthermore, removal of frontal cortex in infancy results in shrinkage of brain size and neocortex thickness as compared with rats receiving similar lesions in adulthood. The current experiment assesses the effectiveness of postlesion experience on the behavior and cortical morphogenesis of rats with frontal cortex ablations at 1, 5, or 10 days of age.

Rats were placed in complex environments on standard laboratory cages at weaning where they were raised until adulthood. They were then given tests of simple motor (postural reflexes, tongue use, gait, etc.), and species typical (grooming, swimming) behaviors as well as a learning test (Morris water task) prior to histological analysis. There were three behavioral effects. (1) The earlier the lesion the worse the animals performed on the behavioral tests, especially the Morris water task. In fact, on this task rats with lesions at 1 day of age performed more poorly than rats with similar removals in adulthood. (2) Environmental enrichment significantly attenuated the behavioral deficit on the learning task and grooming behavior but did not ameliorate the abnormalities in simpler motor tests. (3) Environmental enrichment was most beneficial to those animals with the earliest lesions.

Examination of the cerebral weight, neocortical thickness and cross-sectional area revealed an anatomical effect that paralleled the behavioral results: the earlier the lesion the lighter the brain and the thinner the cortex. Further, environmental enrichment enhanced cerebral weight and cortical thickness in all three age groups.

The results suggest that: 1) very early brain damage may have greater effects upon the animal than similar injury in adulthood; 2) damage at different ages in infancy may have different effects upon the brain; and 3) behavioral therapy may be most effective in attenuating the effects of brain injury when given to those animals with very early lesions.

281.15 EXPERIMENTAL MATERNAL PHENYLKETONURIA IN THE RAT: ENDURING BEHAVIORAL EFFECTS FROM IN UTERO EXPOSURE TO L-PHENYLALANINE (Phe) and p-CHLORO-DL-PHENYLALANINE (pClPhe). A. Rabe, Y.H. Loo*, A. Potempska*, P. Wang* and R. Fersko*. NY State Office of Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Phe and pClPhe, an inhibitor of phenylalanine hydroxylase, have most frequently been used to produce experimental phenylketonuria (PKU). We used these substances to produce experimental maternal PKU. Pregnant Sprague-Dawley rats (n=13) from gestation day 9-20 received s.c. a continuous infusion of 0.2-.45 µmol/g/day of pClPhe and 5.0-7.5 µmol/g/day of Phe with 1mg/100ml of 5HTP added. The infusion rate was 20 ml/24 hr. This schedule was expected to elevate the maternal plasma Phe to 1.7-2.3 µmol/ml and unconjugated phenylacetate (PA, a major metabolite of Phe) to 0.20-0.30 µmol/ml. The control animals (n=13) received physiological saline.

We observed behavioral effects only in litters whose dams' plasma Phe was at least 1.76 and PA 0.21 µmol/ml (n=7), but not lower (n=6). Learning was tested at two different ages, starting on day 17 or day 45, by acquisition and reversal of a left-right position discrimination in a T-maze. The PKU litters (n=7), as compared to the saline litters (n=12), showed an early reversal deficit ($p<0.002$). At 45 days, the PKU litters (n=6) had both an acquisition and a reversal deficit (vs. saline, n=7, $p<.002$). Activity level was measured by ambulation in an open field at 30 days and after 60 days of age. The PKU litters ambulated more at 30 days (PKU, n=7, vs. saline, n=13, $p<.02$), as well as well as after 60 days (PKU, n=5, vs. saline, n=11, $p<.01$).

Since the affected litters came from dams with elevated plasma Phe and PA, the results are consistent with our hypothesis that PA may be the primary agent responsible for brain dysfunction in classical and maternal PKU. However, the present data do not rule out specific effects of Phe and its other metabolites. Although we have shown (Soc. Neurosci. Abstr., 9, 1247, 1983) that infusion of PA during gestation produces rats that also show the same early reversal learning deficit as the Phe+pClPhe treated pups, we still have to determine whether elevated PA alone produces the same lasting learning deficit and activity changes as elevated Phe and PA do.

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281.16 METHYLMERCURY-INDUCED MOVEMENT DISORDERS IN THE NEONATAL RAT: INHIBITION OF GLUTAMIC ACID DECARBOXYLASE IN CEREBRAL CORTEX AND NEOSTRIATUM. J.R. O'Kusky* and E.G. McGeer. Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The toxicity of methylmercury (MeHg) in the human nervous system during prenatal and early postnatal development has been associated with neurological disorders resembling cerebral palsy. Clinical signs in affected children include psychomotor retardation, spasticity, ataxia, athetosis and epileptiform convulsions. Similar signs of neurological impairment have been reported in the neonatal rat following the postnatal administration of MeHg. Neuronal degeneration has been described in the cerebral cortex and neostriatum of these animals at the onset of motor impairment. The present study was conducted to determine the extent to which GABAergic and cholinergic neurons are involved in these neurotoxic lesions.

Three groups of Sprague-Dawley rats received subcutaneous injections of methylmercuric chloride in physiological saline (5 mg/kg) at 24-hr intervals beginning on postnatal day 5 and continuing until one of three stages of MeHg toxicity, defined as follows: Stage I (day 15) when MeHg-treated rats continued to gain weight although less rapidly than normal controls, Stage II (day 20-23) when rats exhibited a loss of body weight, and Stage III (day 23-28) at the onset of neurological impairment. Normal and weight-matched controls were injected with equivalent volumes of saline. The specific activities of glutamic acid decarboxylase (GAD) and choline acetyltransferase (ChAT) were measured in six regions of the central nervous system, including cerebral cortex (frontal and occipital), cerebellum, caudate-putamen, thalamus and spinal cord.

In the occipital cortex there was a significant decrease in GAD activity at Stage II (30%) and Stage III (43%), while GAD activity was reduced only at Stage III in frontal cortex (37%) and caudate-putamen (42%). In the cerebellum, thalamus and spinal cord, GAD activities were normal throughout the experiment. No significant differences in ChAT activity were detected in any of the six regions. These results demonstrate a preferential involvement of GABAergic neurons in MeHg-induced lesions of the cerebral cortex and neostriatum.

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81.PO DEVELOPMENTAL DYSLEXIA: FOURTH CONSECUTIVE CASE WITH CORTICAL ANOMALIES. A.M. Galaburda, G.D. Rosen, G.F. Sherman and F. Aboitiz.* Department of Neurology, Beth Israel Hospital and Harvard Medical School, Boston, MA.

Developmental dyslexia is diagnosed in children with relatively isolated reading difficulties. In the absence of clear biological markers dyslexia includes many children with learning problems affecting other skills as well, and it probably misses patients who have compensated for the reading problem. In recent years attempts have been made to find biological markers of this condition in order to expand knowledge on mechanisms and improve diagnosis and treatment. One such marker may be the presence of anatomical anomalies. A total of 3 brains of dyslexics have been reported. A fourth case will be reviewed here. All four cases have shown cortical anomalies. In the first case, disordered cortical folding and subcortical neuronal rests were seen (Drake, J. Learn. Dis., 1:9, 1968). In the second case, micropolygyria was present on the planum temporale, and there were neuronal ectopias and cortical dysplasias (Galaburda and Kemper, Ann. Neurol. 6:94, 1979). All cortical changes were seen in the left hemisphere. The third case showed large numbers of ectopias and dysplasias predominantly in left hemisphere cortex, with only 4 lesions on the right. The fourth case was that of a 19 year-old right handed dyslexic male with family history of learning disabilities. The patient showed deficits in written language and less severe difficulties with attentional and visuo-spatial tasks in the face of normal intelligence. The brain showed acquired and developmental lesions. The acquired lesions were referable to an episode of trauma. The developmental lesions were those of ectopias, dysplasias and telangiectasia involving the cortex of both hemispheres. The number of lesions was much greater on the left, and on that side involved the superior temporal gyrus and inferior frontal gyrus predominantly. An additional finding is that of symmetry of the planum temporale. Approximately twenty-five percent of unselected autopsy brains show symmetrical plana, whereas in sixty-five percent the left planum is larger (Geschwind and Levitsky, Science, 161:186, 1968). The three dyslexic brains studied in this laboratory have showed a symmetrical pattern.

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CHARACTERIZATION OF PURINE, PEPTIDE, AND AMINO ACID RECEPTORS

282.1 ADENOSINE MEDIATES A SLOW HYPERPOLARIZING SYNAPTIC POTENTIAL (S-H.S.P.) IN CAT VESICAL PARASYMPATHETIC NEURONS. P. Shinnick-Gallagher, T. Akasu* and J.P. Gallagher, Dept. of Pharmacology, The University of Texas Medical Branch, Galveston, TX 77550.

Stimulation (3-10V, 40Hz, 250msec) of preganglionic nerves supplying cat vesical parasympathetic ganglia in the presence of hexamethonium (1mM) produces a muscarinically mediated slow inhibitory postsynaptic potential (s-ipsp). When the stimulus intensity was increased (8-30V), a slower hyperpolarizing potential appeared on the falling phase of the s-ipsp. Atropine (.5-1μM) abolished the s-ipsp and revealed the presence of a s-h.s.p. (2-13mV amplitude; 31.6 ± 5.5 sec duration). This potential was quickly abolished in low Ca/high Mg solution. A s-h.s.p. was discernable with a single stimulus (1 Hz; 1 sec) but maximum amplitude was obtained at 40 Hz.

Adenosine (5μM-1mM) hyperpolarized (5-10mV) most parasympathetic neurons (92%, n=66) whereas ATP (100nM-1mM) depolarized 62% (n=39) of the neurons tested. Caffeine (1mM) an adenosine P_1-purinoceptor antagonist, depressed and subsequently abolished the s-h.s.p. in 11 of 15 neurons. Caffeine also blocked the response to adenosine but not that to ATP. Adenosine deaminase (0.25 IU/ml) which metabolizes adenosine to inosine and NH_3 markedly depressed the s-h.s.p. by 82% (n=5). The adenosine hyperpolarization was similarly depressed 92% (n=7) by 0.25 IU/ml adenosine deaminase. Dipyridamole (3μM) which blocks the uptake of adenosine increased the amplitude (145%; n=6) and duration (185%; n=6) of the s-h.s.p. The amplitude of the adenosine hyperpolarization was similarly enhanced (150%) by dipyridamole (3μM). Both the s-h.s.p. and adenosine hyperpolarization were accompanied by a conductance increase. The amplitude of the s-h.s.p. was decreased by membrane hyperpolarization and reversed polarity beyond -95mV (mean: 93.7 ± 7.4mV; n=5). The adenosine response decreased in amplitude as the membrane was hyperpolarized and reversed polarity at -98mV (n=3). The reversal potential estimated by the intersection of I-V curves determined in control and adenosine (50μM) solution was -93.5 ± 4.3mV (n=9). Both the s-h.s.p. and adenosine hyperpolarization were enhanced in low K^+ and depressed in high K^+ solution, indicating an increase in conductance primarily to K^+ ion. In conclusion, these data provide the first evidence for a synaptic response mediated by adenosine. (Supported by NS16228)

282.2 BINDING OF ^{3}H-NECA TO A SUBTYPE OF A_2 ADENOSINE RECEPTOR IN RAT STRIATAL MEMBRANES. R.F. Bruns, G.H. Lu*, and T.A. Pugsley*. Dept. of Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

^{3}H-NECA (N-ethyladenosine-5'-carboxamide) has been reported to bind to both the A_1 and A_2 subtypes of adenosine (ado) receptors in rat striatal membranes (Yeung and Green, Pharmacologist 23:184, 1981). The object of the present study was to characterize the A_2 component of ^{3}H-NECA binding and compare this receptor to other ado receptors.

Binding was performed using 4 nM ^{3}H-NECA in 50 mM Tris pH 7.7 with 10 mM $MgCl_2$ at 25° in 1 ml with 5 mg wet weight of rat striatal membranes. In initial dose-inhibition experiments, N^6-cyclopentylado (CPA) gave the best separation between A_1 and A_2 sites, with an IC_{50} of 2 nM at A_1 receptors and 700 nM at A_2 receptors. A_1, A_2, and nonspecific binding were respectively 1500, 1500, and 400 cpm. An additional 150 cpm of the nonspecific binding could be displaced by micromolar concentrations of NECA but not by theophylline or CPA. Subsequent experiments used 50 nM CPA to eliminate A_1 binding; nonspecific binding was defined as binding in the presence of 100 μM CPA. To determine A_1/A_2 selectivities, IC_{50}s for compounds in ^{3}H-N^6-cyclohexylado (CHA) binding to A_1 receptors in whole brain were compared to IC_{50}s in ^{3}H-NECA binding to A_2 receptors in striatum. NECA had almost equal affinity at the two receptors (A_1 IC_{50} 11 nM, A_2 IC_{50} 16 nM). The most A_1-selective agonist was N^6-(3-hydroxypropyl)ado (A_1 5 nM, A_2 7,700 nM) and the most A_2-selective agonist was 2-(phenylamino)ado (A_1 1,700 nM, A_2 200 nM). Among the antagonists, 8-cyclopentyltheophylline was the most A_1-selective (A_1 27 nM, A_2 2,800 nM), while alloxazine was modestly A_2-selective (A_1 20 μM, A_2 4.3 μM). A_2 binding of ^{3}H-NECA was highest in striatum, but was detectable at much lower levels in other brain areas.

Daly, et al. (Cell Mol Neurobiol 3:69-80, 1983) proposed that there are two types of A_2 ado receptors in brain: a ubiquitous low-affinity receptor, and a high-affinity receptor localized mainly to the striatum. By the above criteria, the A_2 receptor which is labeled by ^{3}H-NECA in striatum clearly belongs to the high-affinity subtype. Ado analogs with bulky 2-position substitutions appear to differentiate these two A_2 receptor subtypes particularly well: 2-(4-methoxyphenyl)ado has an affinity of 900 nM in ^{3}H-NECA binding, but is inactive at 1 mM at the low-affinity receptor in human fibroblasts. We propose that the high-affinity A_2 receptor be designated A_{2a}, and the low-affinity A_2 receptor A_{2b}.

282.3 FUNCTIONAL RECONSTITUTION OF THE SYNAPTIC MEMBRANE GLUTAMATE-BINDING PROTEIN: GLUTAMATE RECEPTOR-LIKE ACTIVITY IN LIPOSOMES. T.M. Stormann,* H.H. Chang,* K. Johe* and E.K. Michaelis, Depts. Human Development and Biochemistry, Univ. of Kansas, Lawrence, KS 66045.

L-Glutamic acid (L-Glu) interacts with plasma membrane receptors to produce neuronal depolarization. We have purified a glutamate binding protein from rat brain synaptic membranes and from a crude synaptic membrane fraction from bovine brain [Michaelis et al., J. Neurochem. 40, 1742, 1983; J. Neurochem. 42, 397, 1984]. The possible involvement of the glutamate binding protein (GBP) in the activity of synaptic membrane-associated receptor complexes was explored by means of reconstitution into liposomes.

Purification of GBP from rat brain synaptic membranes as described previously and its reconstitution into liposomes, gave a preparation which retained full activity in terms of ligand binding but exhibited small and variable activity as a channel-forming entity responsive to L-Glu. On the other hand, solubilization of synaptic membranes in the presence of an excess of soybean lipids (asolectin, 15 mg/ml) led to functional reconstitution of the synaptic membrane L-Glu-responsive Na^+ channels. Preservation of the activity of the L-Glu-sensitive ion channels was achieved by solubilization with either n-octylglucopyranoside or with Triton X-100 (2% v/v). Reconstitution of the solubilized proteins into liposomes was obtained by incubating these preparations with polystyrene beads. The liposomes that contained synaptic membrane proteins exhibited an L-Glu-enhanced Na^+ flux which was inhibited by the receptor antagonist L-Glu diethylester (10 μM L-Glu and 100 μM L-Glu diethylester). The GBP was subsequently purified in the presence of excess asolectin by affinity batch chromatography on glass fiber with co-reticulated L-Glu according to the procedure described previously (Michaelis, Biochem. Biophys. Res. Commun. 65, 1004, 1975). The protein purified under these conditions could be reconstituted into liposomes with retention of both the L-Glu-binding activity and the L-Glu-initiated Na^+ flux. Electrophoretic analysis of these reconstituted liposomes by SDS-PAGE indicated the presence of a single, small molecular weight protein that migrated to a position identical to that of the purified GBP. (Supported by grants DAAG 29-83-K0065 from the ARO and AA-0439 from the NIAAA.)

282.4 IMMUNOCHEMICAL LOCALIZATION OF THE GLUTAMATE BINDING PROTEIN IN SYNAPTIC MEMBRANES AND ANTIBODY EFFECTS ON GLUTAMATE RECEPTORS. Roy, S. and Michaelis, E.K. Neurobiol. Sect/HDFL, University of Kansas, Lawrence, KS 66045

A small molecular weight glycoprotein that has high affinity binding sites for L-glutamic acid has been purified from rat brain synaptic membranes and from a bovine brain crude membrane fraction. Based on the selectivity of the protein's binding sites for various L-glutamate analogs and on its lack of glutamate metabolizing enzymatic activity, it was proposed that this binding protein may be the recognition macromolecule of the L-glutamate receptor complex. [Michaelis et al., Mol. Cell Biochem. 30, 163- 179, 1981].

Antibodies were raised in rabbits against this purified bovine brain glutamate binding protein [GBP]. Using an Enzyme-Linked Immunosorbent Assay [ELISA] the anti-GBP antibodies were found to be highly specific for the bovine GBP and the analogous protein purified from the rat with little or no cross-reactivity against glutamate-metabolizing enzymes [Roy and Michaelis, J. Neurochem. 42, 838-841, 1984]. We report here the use of these GBP antibodies to localize and quantify the GBP content in various subcellular fractions obtained from rat brain. Our results show that the distribution of the immunoreactivity followed a similar pattern of quantitative enrichment in brain subfractions as that observed for L-glutamate binding. The order of immunoreactivity was: synaptic membranes > crude mitochondrial fraction > homogenate > myelin.

Specific anti-GBP antibodies were purified using an affinity chromatographic procedure. The purified antibodies were then tested for their activity as inhibitors of glutamate binding to the rat brain GBP and of glutamate-induced SCN^- flux. The latter is a measure of depolarization brought about by glutamate activation of synaptic membrane receptor-ion channel complexes [Chang and Michaelis, Biochim. Biophys. Acta. 688, 185-294, 1982]. The specific anti-GBP antibodies were found to block 76% of the glutamate binding to the GBP at concentrations of 25-50 ng IgG/0.1 ml and to inhibit totally L-glutamate-induced SCN^- flux at amounts of 1 μgIgG/mg protein. The purified antibodies exhibited 20-fold or greater inhibitory activity as compared with the antiserum or the whole IgG fraction. (Supported by grant DAAG-29-83K-0065 from ARO and KS-83-73 from the American Heart Association.)

282.5 MONOCLONAL ANTIBODIES AND PEPTIDE MAPPING REVEAL STRUCTURAL HOMOLOGIES BETWEEN THE SUBUNITS OF THE GLYCINE RECEPTOR OF RAT SPINAL CORD. H. Betz, F. Pfeiffer*, R. Simler* and G. Grenningloh*. (SPON: ENA). Institute for Neurobiology, ZMBH, Universität Heidelberg, Im Neuenheimer Feld 364, D-6900 Heidelberg, Federal Republic of Germany.

The glycine receptor of rat spinal cord is an oligomeric membrane glycoprotein of molecular mass 250,000 daltons which contains three polypeptides of 48,000, 58,000, and 93,000 daltons (Pfeiffer et al., J. Biol. Chem., 257,9389, 1982). The strychnine binding site of the glycine receptor has been localized on the 48,000 dalton subunit (Graham et al., Eur. J. Biochem., 131,519, 1983).

Monoclonal antibodies (mAbs) were prepared against the affinity-purified glycine receptor protein using ^{125}I-labeled receptor preparations for the detection of positive hybrids. From nine monoclonal antibodies obtained, six recognized denatured receptor polypeptides blotted to nitrocellulose paper. Two of these antibodies bound to more than one glycine receptor subunit: mAb GlyR 4a stained the 48,000 and 58,000 dalton polypeptides, and mAb GlyR 7a the 48,000 and 93,000 dalton polypeptides. Common antigenic determinants thus are shared by the different subunits of the glycine receptor.

Complementary results were obtained by peptide mapping of ^{125}I-labeled glycine receptor polypeptides with various proteases. A set of peptide fragments of the same apparent molecular weight was produced from the different glycine receptor subunits using V8-protease, chymotrypsin, and elastase. These data suggest that the three subunits of the glycine receptor have considerable homology within their primary structure and may have evolved from a common ancestor receptor polypeptide.

This work was supported by the Deutsche Forschungsgemeinschaft, the Stiftung Volkswagenwerk and the Bundesministerium für Forschung und Technologie.

282.6 THE SIGMA OPIOID RECEPTOR: CHARACTERIZATION AND CO-IDENTITY WITH THE PHENCYCLIDINE RECEPTOR. L. G. Mendelsohn, Vin Kalra* and Bryan G. Johnson*. The Lilly Research Laboratories, Eli Lilly and Company, 307 East McCarty Street, Indianapolis, Indiana 46285.

The properties of the sigma opioid receptor of rat brain cortex have been characterized using the prototypic ligand [^3H] (+) SKF 10,047. Binding to this receptor was rapid, and equilibrium was obtained within 30 min at 37°. Specific binding was linear with protein concentration up to 500 μg/2 ml and was dependent upon protein integrity. Denaturation by boiling destroyed over 95 percent of the specific binding. A high affinity binding site was identified through Scatchard analysis with a K_D of 151.3 ± 43.2 nM and a maximum binding of 2.91 ± 0.84 pm/mg protein. The addition of salt, either NaCl or $CaCl_2$, to the buffers markedly decreased binding, with $CaCl_2$ being more potent than NaCl. A broad pH optimum for specific binding was observed; maximum binding was at pH 9.0. The affinity of a number of ligands for the sigma site and the PCP receptor were compared. The binding (IC_{50}) of 15 ligands to the sigma site showed a correlation of 0.842 ($p < 0.001$) with binding to the PCP site. The data demonstrate that the biochemical properties of the sigma and PCP receptors are similar and support the view that these receptors are one and the same site.

282.7 COMPARATIVE RECEPTOR BINDING PROPERTIES OF [^{3}H]TCP AND [^{3}H] DEXOXADROL, TWO PHENCYCLIDINE (PCP)-RELATED LIGANDS. C. Pilapil and R. Quirion. Douglas Hospital Research Centre, Verdun, Quebec, Canada H4H 1R3.

Various groups have described the existence of specific PCP binding sites in brain. However, because of the relatively poor affinity (K_I = 50-200 µM) of PCP itself, it has not been possible to very precisely characterize these sites. Vignon et al (Brain Res. 280:194-197, 1983) have recently demonstrated that [^{3}H]TCP, a potent analogue of PCP, could be a more suitable ligand to study brain PCP binding sites. We have characterized receptor binding properties of TCP, as well as dexoxadrol, a general anesthetic with potent PCP-like actions in behavioral paradigms. Rat brain membranes were prepared as described before (Quirion et al, Peptides, submitted) and then incubated for 60 min in 5.0 mM Tris.HCl, 50 mM sucrose pH 7.4 at 4°C in presence of various concentrations of [^{3}H]TCP or [^{3}H]dexoxadrol. Specific binding was defined as radioactive ligands bound in presence and absence of 100 µM PCP or 10 µM dexoxadrol. Filters used in assays were presoaked in 0.1% polyethyleneimine at least 2-3 hrs before filtration to reduce binding of ligands to filters. Under these conditions, [^{3}H]TCP labels an apparent single class of sites with a K_d of 5.6 nM and a B_{max} of 583 fmol/mg protein. Ligand selectivity pattern shows that PCP (IC_{50} = 179 nM) > cyclazocine (232 nM) > (+) SKF 10,047 (537 nM) > (-) SKF 10,047 (> 2000 nM). On the other hand [^{3}H] dexoxadrol appears to label two classes of sites. [^{3}H] dexoxadrol binding to the high affinity site (K_d = 2.8 nM; B_{max} = 150 fmol/mg protein) is not displaced by PCP and sigma opiate agonists. The lower affinity site (K_d = 9 nM) is much more abundant with a B_{max} of 701 fmol/mg protein and a ligand selectivity pattern showing that dexoxadrol (14 nM) > PCP (359 nM) > (+) SKF 10,047 (987 nM). Thus, [^{3}H]dexoxadrol appears to label two sites: a high affinity site that remained to be characterized and a lower affinity site that is likely to be PCP-related. Our data demonstrate that [^{3}H] TCP and [^{3}H]dexoxadrol show important differences in their interactions with putative PCP receptor binding sites.

282.8 MEASUREMENT OF ADENOSINE (A_1) RECEPTORS IN RATS FOLLOWING REM SLEEP DEPRIVATION. G. Yanik, N.M. Porter*, R.D. Green, and M. Radulovacki. Dept. Pharmacology, University Illinois College of Medicine, Chicago, IL 60612

Recent studies done in this laboratory have shown that administration of adenosine and its congeners at certain doses increased deep slow wave sleep and REM sleep in rats (Radulovacki et al., JPET 228: 268, 1984; Virus et al. Neuropharm. 22: 1401, 1983). However, the role of endogenous adenosine in sleep remains unclear. The aim of the present study was to assay adenosine (A_1) receptors in specific brain structures of rats deprived of REM sleep for 48 hours.

Male Sprague-Dawley rats, 300-350 g, were deprived of REM sleep utilizing the "flower-pot" method (Mendelson et al., Pharmacol. Biochem. Behav. 2: 543, 1974) for 48 hours. Control and REM sleep deprived (RD) groups were given ad libitum access to food and water for a period of 15 min each 12 hrs during the experiment.

At 48 hrs animals were killed by decapitation and their brains were rapidly removed and dissected to remove the following structures: cortex, striatum, hippocampus and cerebellum. All brain samples were kept frozen at -70°C until analyzed. Adenosine A_1 receptor binding was assayed using H^3L-PIA.

		CTX	HIP	STR	CER
C	K_D(nM)	2.9 ± .4(11)	3.3 ± .5(12)	4.1 ± .5(9)	2.6 ± .6(3)
	Bmax(fm/mg)	511 ± 25	745 ± 37	378 ± 20.5	848 ± 73
RD	K_D(nM)	4.2 ± .6(12)	4.9 ± .7(12)	5.2 ± .5(10)	3.6 ± 1.4(4)
	Bmax(fm/mg)	587 ± 21*	714 ± 36	466 ± 26.8*	783 ± 104

All values are means ± S.E.M.; Numbers in parentheses indicate N for group *$P \leq .05$ as determined by student's t test for independent measures. K_D expressed as nanomolar and Bmax as fmole/mg protein.

As can be seen in the table, a statistically significant increase in Bmax was found in cortex and striatum of REM deprived animals. In addition, preliminary results from this laboratory suggest that adenosine levels in specific brain structures taken from animals killed by microwave irradiation after total sleep deprivation, and also following 24 or 48 hour of RD showed no statistical change from control adenosine levels in any structure analyzed. The structures included: frontal cortex, occipital cortex, thalamus, hippocampus, striatum, and hypothalamus (method of Wojcik and Neff, J. Neurochem. 39(1) 280, 1982). These data taken together suggest that the actions of endogenous adenosine on sleep may be due to receptor mediated changes and not due to increases in adenosine levels. (Supported by ONR Contract N000 14-79-C-0420)

ALCOHOL AND BARBITURATES II

283.1 BLOOD ETHANOL CONCENTRATIONS AND ETHANOL-INDUCED CHANGES IN THE AUDITORY BRAINSTEM RESPONSE. J. A. Lee#~, R. F.Berman~, D. W. Nielsen,~#, W.-N. Lin*^ and A. R. Kelly#^. ~Psychology Department, Wayne State University; #Otological Research Laboratory, and ^Pharmacology and Toxicology Division, Henry Ford Hospital, Detroit, MI 48202.

Alcohol affects the Auditory Brainstem Response (ABR) in a dose-related fashion. Squires, Chu, and Starr (Electroenceph. clin. Neurophysiol, 45:577, 1978) and Lee, Berman, Nielsen, and Schoener (Neurosci. Abstr., 8:595, 1982) found ABR wave slowing 45 min following a moderate, 2.5 g/kg, ethanol dose. Lee, et al (1981) also found prolonged wave slowing that began immediately following a high dose of ethanol (5.0 g/kg). Moreover, with the moderate dose they found an excitatory effect (decreased wave latencies) that preceeded the wave slowing, which started 45 min after alcohol. It was suggested that the excitatory effect might correspond to the rising phase of blood alcohol levels. This study was undertaken to determine blood ethanol concentrations following ethanol administered under conditions identical to those during the ABR alcohol dose-effect study.

Male Long-Evans rats were prepared with chronic aortic catheters. Rats were 16-20 hrs food deprived at the time of ethanol administration. Ethanol doses, 5.0, 2.5, and 0.5 g/kg, were administered by gavage in equal volume. Blood was sampled at given intervals over 2 1/2 hrs, beginning 2 min after ethanol administration. Samples were collected in 70 ul hematocrit capillary tubes, and blood ethanol concentrations were determined by gas chromatography.

Blood ethanol curves for the three doses differed according to absolute ethanol concentration, rise time, peak time, and slope of the linear falling phase. The highest peak and the steepest linear falling phase occurred for the highest dose. The moderate dose produced the longest rise time, peaking later than both the low and high doses. The low dose blood ethanol curve-ABR association was inconclusive. The excitatory effects following the moderate dose corresponded to the rising and peak phases; the onset of ABR wave slowing coincided with the beginning of the linear falling phase. For the high dose, ABR wave slowing was associated with every phase of the blood ethanol curve. According to these results, ABR excitatory effects are associated with rising and peaking phases of blood alcohol concentration when the rise is not too steep and of appropriate height. ABR wave slowing is associated with the linear falling phase and with fast rising and peaking phases.

283.2 TOLERANCE DEVELOPMENT TO ETHANOL INDUCED HYPOTHERMIA: ROLE OF BEHAVIORAL RESPONSES. R. Spencer*, P. Marques*, S. Hsiao and T. Burks (SPON: P. Pickens). Depts. of Psychology and Pharmacology, Univ. of Arizona, Tucson, AZ 85724.

Acute ethanol (ETOH) lowers body temperature of rats at room temperature. Development of tolerance to ETOH induced hypothermia has been demonstrated. However, the effect of ETOH on behavioral thermoregulation, as tolerance develops, has not been previously characterized. We have devised a system which allows for the simultaneous measurement of core body temperature (Tc), selected "preferred" ambient temperature (Tp) and general motor activity of rats. Tp was monitored by placing rats in a 2 m long, 10 cm wide opaque hollow plexiglass tube in which a linear temperature gradient (5°C-35°C) was established. Tc was transmitted by a temperature sensitive telemetry capsule placed in the peritoneal cavity. The range and frequency of movement within the tube provided a measure of motor activity. For 14 consecutive days Tc, Tp and activity levels were monitored in male albino Sprague-Dawley rats (200-250 gm) following i.p. injections of 2.5 gm/kg ETOH (20% v/v in saline) or an equivalent volume of saline for control rats. On day 15 all rats received a "placebo" saline injection. On day 1, ETOH treated rats exhibited a mean peak hypothermia of -1.24°C compared to controls. By day 14 there was no significant difference in Tc between ETOH and saline injected rats. Mean hypothermia for ETOH treated rats diminished in a linear fashion over the 14 days (r=-.91). Following a "placebo challenge" on day 15, the ETOH tolerant rats exhibited a mean .72°C hyperthermic response compared to controls. The ETOH injected rats selected a cooler and more variable Tp than control rats throughout the 14 day period (mean of 18.3°C for ETOH vs. 22.6°C for saline). There was a trend for increasing mean Tp for the first 20 min after ETOH injection over the 14 days (mean Tp for days 1-5 = 14.9°C and mean Tp for days 10-14 = 18.2°C). Activity levels of ETOH treated rats were significantly depressed for the entire 14 day period. Thus, although rats became totally tolerant to the hypothermic effects of ETOH, they did not become tolerant to the activity depressant effects. There was evidence for behavioral thermoregulatory adjustments over the 14 days, however, the magnitude and consistency of change was not nearly as dramatic as the change in peak hypothermia. These results suggest that tolerance to the hypothermic effects of ETOH are more attributable to autonomic thermoregulatory responses than to behavioral thermoregulatory responses.

283.3 THE EFFECT OF SHORT-TERM ETHANOL FEEDING ON PITUITARY LUTEINIZING HORMONE BIOACTIVITY. M. Emanuele*, K. Ward*, R. Petrutis*, L. Wallock*, L. Kirsteins*, N. Emanuele* and A.M. Lawrence* (SPON: J.A. McLane). Biochem. Neuroendocrinol. Research Lab., VA Hospital, Hines, IL 60141, and Depts. of Medicine and Biochemistry, Loyola University Stritch School of Medicine, Maywood, IL 60153.

Excess ethanol (ETOH) exposure is known to exert a deleterious effect on reproductive processes in man and animals. Although serum levels of luteinizing hormone (LH) differ between control and ETOH-exposed animals, there is no information as to whether the bioactivity of pituitary LH differs. In this study, twenty Sprague-Dawley male rats either received a liquid ETOH diet or were pair-fed isocalorically with dextrimaltose substituted for the ETOH. In addition, five animals were fed rat chow ad libitum. Two weeks later, all animals were sacrificed by decapitation and serum and pituitary LH was measured by a validated radioimmunoassay. Basal serum LH levels were undetectable in both the ETOH and pair-fed groups of rats but detectable in the rat chow group. Pituitary LH levels were significantly higher in the ETOH-fed compared to the control-fed group (762 ng/ml vs 502 ng/ml; $p < .001$). When LH extracted from control-fed and ETOH-exposed pituitaries was assayed in a rat testis interstitial cell testosterone secretion (RICT) bioassay, it was shown that pituitary LH from the ETOH-fed group promoted significantly less testosterone secretion per ng LH compared to the secretion of testosterone that was evoked by immunoequivalent amounts of pituitary LH from the pair-fed animals. Conclusion: Short-term ETOH feeding to adult male rats was associated with a significant decrease in the bioactivity of pituitary LH as assessed by LH-induction of testosterone secretion from Leydig cells in vitro. A significant increase in total immunoreactive LH content was also found in pituitaries from ETOH-exposed animals; this finding, coupled with the undetectable serum LH levels, may indicate a problem with pituitary LH release in the presence of ETOH. The finding of undetectable serum LH in the ETOH and pair-fed animals may relate to subtle nutritional deficiencies since serum LH was measurable in the group that received rat chow ad libitum.

283.4 EFFECTS OF ACUTE AND CHRONIC ETHANOL TREATMENT ON THE ELECTROTONIC PARAMETERS OF HIPPOCAMPAL NEURONS. D. Durand and P.L. Carlen. Applied Neural Control Lab., Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OHIO, Addiction Research Foundation and Playfair Neuroscience Unit, Toronto, ONTARIO.

Chronic ingestion of ethanol has been shown to produce psychological, behavioral, and morphological damage. The hippocampus is particularly affected and some of the pathophysiological effects have recently been investigated. Both extracellular and intracellular recordings have shown a significant impairment of the inhibitory mechanisms. However, very little has been published on the effect of ethanol on the passive electrical properties of nerve cells and both the chronic and the acute effects have been investigated in-vitro.

For 5 months, male Sprague Dawley rats were provided ad-libitum but measured access to a liquid diet containing 35% of its calories as ethanol while a control group received the same diet with ethanol replaced by maltose-dextrins. The ethanol group was withdrawn for 3 weeks before the intracellular recordings of granule cells were obtained. Short current pulses were injected into the neurons and the resulting voltage decays analyzed with a computer model of granule cells. The model consisted of an equivalent dendritic cylinder coupled to a soma and a somatic shunt. The following parameters were estimated: electrotonic length L, dendritic dominance ratio ρ, somatic shunt coefficient ε, membrane time constant τm, input resistance Rn, the axoplasmic specific resistance Ri, the membrane specific resistance Rm and capacitance Cm. The results showed no significant difference in τm, Rn, ρ and ε. However, the following parameters were significantly affected as shown in the table:

	Control	N	Ethanol	N	Prob
L	.85	22	.94	22	.05
Rm (Ohm.cm2)	6632	9	18460	7	.01
Cm (uF/cm2)	4.48	9	1.37	7	.02
Ri (Ohm.cm)	147	7	54	7	.05

Acute ethanol administration on CA1 neurons suggested that ethanol increased membrane capacitance. In 7/12 cells using 20mM ethanol and in 6/6 using 100mM ethanol, an increased τm (up to 75%) resulted with little change or decrease in Rn, suggesting in these cells that ethanol increased membrane capacitance. The relationship between a decrease in Cm produced by chronic ethanol and the increase in Cm produced by acute ethanol remains to be investigated.

283.5 DIFFERENTIAL EFFECTS OF ETHANOL AND OPIATES ON BSR THRESHOLD, M.J. Lewis, J.R. Andrade,* C.Mebane*, and R. Phelps* Dept. of Psychology, Howard University, Washington, D.C. 20059

The rewarding effects of both ethanol (ETOH) and opiates are widely believed to play a vital role in their self-administration and abuse. Based on research that shows that condensation products of monoamine neurotransmitters with the alcohol metabolite acetaldehyde 1. have opiate-like effects, 2. may enhance ETOH self administration, and 3. may be produced endogeneously with chronic ETOH administration, it has been suggested that opiates and ETOH may have a common substrate of reward. In the study reported here, the reinforcing properties of both drugs were investigated using brain stimulation reward (BSR) in laboratory rats.

Male albino rats were implanted with platinum bipolar electrodes using standard stereotoxic procedures. Electrodes were directed at lateral hypothalmic (LH) sites within the medial forebrain bundle or the ventral tegmentum (VT) posterior to the substantia nigra. Animals were shaped to lever-press for BSR at each site. Animals were trained initially under continuous reinforcement and then under a fixed ratio schedule of reinforcement. Thresholds for BSR were determined using a modification of the technique of Huston and Mills (1971) (Phelps and Lewis, Behavior Research Methods and Instrumentation, 1982, 14 (3) 323-328).

Morphine (2.5 and 5 mg/mg, ip) lowered BSR threshold in both the LH and VT. The opiate-antagonist naloxone (1.6 and 5.0 mg/kg) increased threshold in the VT, but not in the MFB. Conversely ETOH (.25 and .50 gm/kg lowered BSR threshold at LH sites, but not in the VT.

These data suggest that at low doses, these drugs do not share a common substrate of reward. (Supported in part by AA06263 and DA02176).

283.6 ALCOHOL-INDUCED "MANIC" RUNNING IN MICE. R.J. Douglas*, E.T. McGarvey* & G.M. Clark. Psychology Dept., Univ. Washington, Seattle, Wash., 98195.

In an automated apparatus we continuously monitor activity in terms of photocell beam breaks. "General activity" consists of beam breaks in any sequence whereas "locomotion" refers to a foreward progression of beam breaks. Mice can engage in pure locomotion by running along either of two circular paths. The time course of drug action is observed by placing mice in the apparatus immediately after injection. Of the many drugs tested so far, ethanol and pentobarbital both specifically stimulate locomotion, but we have concentrated on the effects of i.p. injections of 10% ethanol in saline. Mice are usually begun at a dose of .25 g/kg, which is incremented by .25 on successive days up to a maximum of 2 g/kg. Peak locomotion usually occurs between 1.0 and 1.75 g/kg, with higher doses producing severe ataxia and/or stupor. At the optimal dose virtually all behavior is channeled into locomotion, and the running behavior could be described as "manic" or "frenzied". Speeds of 25 cm/sec have been maintained for over 15 min. Peak locomotion often coincides with some degree of hindlimb ataxia. Catecholaminergic stimulants, in contrast, may increase general activity, but they actually reduce locomotion. These drugs also detract from the locomotor stimulant effect of alcohol, in support of the observations of Abbott & Dudek (Neurosci Abstr 7:313, 1981).

Drugs affecting the cholinergic system have potent effects when employed as pretreatment for alcohol injection. Scopolamine, for example, potentiates the locomotion-stimulating effect of alcohol while the cholinergic stimulant physostigmine blocks this effect at doses as low as .05 mg/kg. The effects of polyethylene glycol (POLY) are even more striking. We use a 25% solution of POLY in saline and have found the drug, when used by itself, to have no consistent behavioral effects at doses up to 16 g/kg. When employed as pretreatment for alcohol, however, a dose of 5 g/kg can completely block alcohol-induced locomotion when alcohol is injected 30 min later and can greatly reduce the effects of alcohol injected up to a week later. POLY pretreatment also appears to greatly reduce ataxia and other signs of alcohol inebriation.

283.7 ETHANOL, HYPOTHERMIA AND SUSPENDED ANIMATION IN MICE: PRELIMINARY STUDIES. R.L. Alkana, G.G. Galleisky*, M. Bejanian, P.J. Syapin and D.A. Finn*. Inst. for Toxicology, School of Pharmacy, Univ. of So. Calif., Los Angeles, CA 90033.

Incidental observations in our laboratory suggested that lowering the body temperature of ethanol intoxicated mice below 25 °C by exposing them to cold environments markedly slowed metabolic activity as measured by weight loss and respiratory rate. These animals were not responsive behaviorally, but recovered normal function after warming. The present study attempted to replicate these findings under more controlled conditions. Adult, drug-naive, weight matched, male C57BL/6J mice were injected i.p. with 5.0g/kg ethanol (20% w/v) or with normal saline and placed into chambers at 15 or 22 °C without food or water for 24 hours after injection. Body weights, rectal temperatures, respiratory rates and general behavior were recorded. The ethanol injected mice exposed to 15 °C lost significantly less weight (3.4%) than the ethanol injected mice exposed to 22 °C (18.6%) and saline injected mice exposed to 15 °C (24.8%) and 22 °C (22.0%). The body temperature of ethanol injected mice exposed to 15 °C dropped to 16 ± 1.0 °C by 4 hours post injection and remained at that level for 24 hours. In contrast, the rectal temperatures of ethanol injected mice exposed to 22 °C had returned to normal by 24 hours post injection. The body temperatures of saline injected animals did not change significantly over the 24 hour period regardless of the ambient temperature to which they were exposed. The respiratory rate of ethanol-cold exposed mice dropped from approximately 190 breaths per minute (bpm) prior to treatment to < 80 bpm at 4 hours and was further reduced to < 30 bpm at 24 hours post injection. Respiratory rates in the ethanol 22 °C and saline treated animals were not significantly different at 4 and 24 hours after injection than they were before treatment. The marked reduction in weight loss and respiratory rate in the intoxicated 15 °C exposed animals suggests that their metabolic rate had been reduced by over 80% in comparison with mice receiving an equivalent amount of ethanol-derived calories and exposed to 22 °C. These results agree with previous work indicating that controlled hypothermia can be used to protect tissues from the destructive effects of anoxia and extends these findings to suggest the exciting possibility that ethanol might represent a tool for inducing prolonged suspended animation or artificial hibernation (supported by USPHS research grant RO1 AA05234, NIAAA, ADAMHA).

283.8 A COMPARISON BETWEEN RECTAL AND BRAIN TEMPERATURES IN ETHANOL-INTOXICATED MICE. M. Bejanian, D.A. Finn*, P.J. Syapin, D. Boone*, and R.L. Alkana. Inst. for Toxicology, School of Pharmacy, Univ. of So. Calif., Los Angeles, CA 90033.

In previous studies, our laboratory has used deep rectal temperature as a substitute for measuring brain temperature in intoxicated mice exposed to different ambient temperatures. The present study investigated the accuracy of this substitution. Adult, drug-naive, male C57BL/6J mice were injected i.p. with 4.0 g/kg ethanol (20% w/v) or normal saline, caged individually and placed inside a temperature controlled chamber at 15, 22, 32, or 34 °C. Thirty minutes post-injection the animals were removed from the chamber. Their rectal temperatures were taken. Then, they were killed by cervical dislocation, decapitated and a thermister probe (Bailey Instruments, Model RET-3) was inserted through the foramen magnum into the third ventricle. The rectal temperature of the body stump and the brain temperature were recorded simultaneously for 15 seconds. Rectal and brain temperatures in the intoxicated mice were highly correlated (r^2 = 0.90) with both temperatures increasing significantly as the ambient temperature increased. However, brain temperature increased more slowly than did rectal temperature and was significantly lower than rectal temperature in intoxicated mice exposed to 32 and 34 °C but was not significantly different than rectal temperature in mice exposed to 15 and 22 °C. In sober mice, brain temperature was always significantly lower than rectal temperature and these temperatures remained relatively constant regardless of the ambient temperature. These results indicate that rectal and brain temperatures are highly correlated in intoxicated mice, but they do not change in parallel during ambient temperature challenge. Therefore, rectal temperature *per se* provides only a rough estimate of the effect of ambient temperature challenge on brain temperature in intoxicated mice. However, rectal temperature can be used to calculate a highly accurate estimate of brain temperature in these animals if the relevant regression information is available (Supported by USPHS grant RO1 AA05234, NIAAA, ADAMHA).

283.9 AMPHETAMINE AND NICOTINE INCREASE ETHANOL INTAKE: A STRESS REDUCTION MODEL? A.D. Levy, M.M. Morgan, & G. Ellison. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

We have previously demonstrated that continuous low-level administration of amphetamine (AMPH) or nicotine increases preference for ethanol in rats (Pharm Bio Beh 18:489). Both nicotine and AMPH increase corticosterone (CORT) levels (Psychopharm. 63:7; Neuroendocrin. 29: 110) suggesting that these agents act as stressors. To determine whether the increased ethanol intake represents stress reduction, daily locomotor activity, as well as CORT, ACTH, and adrenal weights were measured in rats receiving continuous AMPH, nicotine, or vehicle, and given access to both ethanol and water, or water only.

Rats housed in tilt-cages for daily locomotor activity measurements, were given ad lib access to water, 10% ethanol, and food for at least 30 days. Pellets for continuous administration of AMPH, nicotine or vehicle were implanted subcutaneously. Twelve days after pellet implantation, all animals were sacrificed by decapitation, and blood samples were collected for radioimmunoassay of CORT and ACTH. Adrenals were also removed and weighed.

Intake of ethanol was increased from baseline levels following AMPH or nicotine pellet implantation, but was unchanged in control pellet groups. Activity was increased by AMPH administration, and remained high throughout the 12 days. Nicotine slightly reduced locomotor activity. Ethanol intake did not significantly alter activity in the control pellet group, nor did it alter the effects of AMPH or nicotine on activity. Adrenal weights were increased in groups receiving AMPH or nicotine pellets, and were not attenuated in groups with access to ethanol. Locomotor activity does not appear to be an important factor in the drug-induced increase of ethanol intake. Assay of CORT and ACTH levels will be discussed in terms of whether continuous drug administration acts as a stressor, and the degree to which the increased ethanol intake antagonizes the stress.

283.10 ETHANOL SELF-ADMINISTRATION IN RATS: EFFECTS OF ACUTE AMPHETAMINE. A.O.Pfeffer* & H.H.Samson* (SPON: D.Finocchio). Dept. of Psych. Univ. of WA, Seattle, WA 98195.

The role of catecholamines in mediation of drug self-administration has received much attention in recent years. However, there have been no studies of the effects of psychomotor stimulants on oral ethanol self-administration. The present study employs an operant technique to examine the effects of dl-amphetamine (AMP) on ethanol as a scheduled reinforcer.

Six male Long-Evans rats, maintained at 80% body weight by food restriction, were trained to leverpress for water and 5% (v/v) ethanol on a concurrent FR8-FR8 schedule. After leverpressing rate in daily 30-minute sessions had stabilized, i.p. injections were given 30 minutes before sessions, as follows. On Mondays and Fridays no injections were given. On Tuesdays and Thursdays drug vehicle and on Wednesdays either 0.25, 0.5 or 1.0 mg/kg doses of AMP were injected. Each rat received each AMP dose twice.

In baseline, all rats prefered ethanol to water (median ethanol preference = 90%). Neither vehicle nor AMP had any effect on water responding. The low dose of AMP significantly ($p<.05$) increased responding for ethanol (mean increase = 21%, sd= 42%) and the high dose significantly ($p<.025$) decreased responding for ethanol (mean decrease = 66%, sd = 43%). Neither the vehicle nor the middle dose of AMP had a significant effect on responding for ethanol. Lack of significance under the middle dose was due to heterogeneity of AMP effect: two rats increased responding, two decreased, and two increased on one drug injection and decreased on the other. Baseline rate of leverpressing showed no relation to AMP effects.

Several possible interpretions exist. First, AMP may simply increase arousal and thus increase responding, until at the higher doses stereotypic behaviors interfere with scheduled leverpressing. Casual observation of some rats at the highest AMP dose revealed the presence of stereotypic movement patterns. Second, the amount of DA that is released by a low dose of AMP may potentiate other reinforcers, mediated by the mesolimbic DA system. A larger AMP dose could saturate DA receptor sites, and render reinforcers ineffective. Third, AMP is known to affect both DA and NE systems. If the two systems are involved differentially in arousal and reinforcement, at different doses the combined effects may be manifested differently.

283.11 HYPOTHALAMIC ETHANOL APPETITE CENTER STUDIED BY MEANS OF EtOH FREE SELECTION (E.F.S.); INHERITANCE IN "DRINKER" & "NON DRINKER" RAT. E. Egaña, *F. González, *M. Labbé, *M. Larragibel &*H. Muñoz. University of Chile - Faculty of Medicine - Institute of Experimental Medicine - Laboratory of Neurochemistry; Santiago 7 - Chile.

It is accepted that E.F.S. is a reliable method to study EtOH appetite hypothalamic "center". Also to study the eventual inheritance of EtOH addition ("Drinker & Non Drinker mammals"). We report experiments on E.F.S. in normal adult ♂ & ♀ rats in which EtOH of diverse concentration were offered in addition to H_2O: 3%,12%,18% & 25% v/v. Individual cages, calibrated vessels rotated dayly. Results as g EtOH/100 g rat/24 h. Lapse:35-40 days. N° animals: ♂ 110 & ♀ 105. According to EtOH dayly consumption the rats were clasified:D1=≥ 0.80;D2=0.75-0.65;D3=0.60-0.50;D4=0.45-0.35 D5=0.30-0.20;D6=≤ 0.20.Crossed ♂+♀ of identical D. See TABLE 1.

TABLE 1
E.F.S., "DRINKER" &"NON DRINKER", % IN EACH GENERATION

♂ 1st Generation						♀					
D1	D2	D3	D4	D5	D6	D1	D2	D3	D4	D5	D6
32.5	23.3	33.2	11.0	—	—	28.7	27.3	44.0	—	—	—
2nd Generation											
31.0	28.4	33.1	7.5	—	—	25.8	28.2	42.9	3.1	—	—
3rd Generation											
36.2	20.2	31.9	10.7	1.0	—	47.0	16.8	30.5	5.7	-	-

N= Normal adult rat (110 ♂ & 105 ♀). 1st generation 60, 2nd generation 76; 3rd generation 79.

There was no significant difference between ♂ & ♀ .Higher % found in D1 & D3 particularly in ♀ 2nd & 3rd generations. ♂ preferred more dilute (3% & 12% v/v EtOH) & ♀ choose higher concentration (18% & 25% v/v).The 3 generations showed similar percentage of D, with minor variations particularly D1 & D3. D5 & D6 were not found (except ♂ 3rd generation). A preliminary conclusion could be that hypothalamic EtOH-appetite "center" is selective regarding the EtOH concentration intake, in the sense that high D could be got by drinking either 3% or 25% EtOH sol. These findings may favor the inheritance theory of alcohol addition in mammal (including human), process which could arrise from an enzime-mutation or permanent enzime-adaptation in neurons of the EtOH appetite "center" which envolves changes of DNA/RNA/protein function.

*Research student Faculty of Medicine. Grant Project B 1050-845-5 by D.I. University of Chile.

283.12 NIGRAL & SYSTEMIC GABA AGONISTS: EFFECTS ON EVOKED FIELD POTENTIALS DURING ETHANOL WITHDRAWAL. L.P. Gonzalez and M.K. Hettinger. Alcohol and Drug Abuse Res. and Training Program, and Dept. of Physiol. and Biophys., Univ. of Ill. at Chicago, Hlth. Sci. Cntr., Chicago, Il. 60612.

We have recently reported results which suggest that GABAergic neurons of the substantia nigra may be regulators of ethanol (EtOH) withdrawal seizures and related symptomatology (Brain Res., 1984, 298:163-166). In those studies, we observed a significant suppression of induced motor convulsions following intranigral administration of the GABA agonist muscimol. In order to further evaluate the role of GABA-receptive neurons in the EtOH withdrawal syndrome, we examined the effects of local injections of muscimol and systemic injections of diazepam on withdrawal seizures, and the associated changes in cortical and subcortical neuronal activity. Sprague-Dawley rats received chronic bilateral implants of guide cannulae placed just above the substantia nigra zona reticulata (SNR). Monopolar, semimicroelectrodes were implanted within the amygdala, hippocampus, and SNR, and bilateral electrodes over motor and visual cortex. One week after surgery the animals received chronic EtOH exposure in EtOH-vapor inhalation chambers. Following ten days of chronic exposure, the animals were removed from the chambers and were observed for evidence of withdrawal hyperexcitability. Eight hrs after removal from the chamber, a series of photic-stimulated field potentials (FP's) were recorded from the implanted electrodes. Animals then received either bilateral injections (0.5 ul) into SNR of either saline or muscimol (15.0 or 30.0 ng per side), or systemic injections of saline (1 cc/Kg) or diazepam (1.25 or 2.50 mg/Kg). Twenty min after injection, another series of FP's were recorded and the animals were then tested for susceptibility to audiogenic seizures.

Both intranigral muscimol and systemic diazepam were found to significantly inhibit seizures. Both treatments also increased the latency of late FP components and reduced the amplitude of FP's at all subcortical sites in a dose-dependent manner. The greatest effects were seen in late components and in the response to a second stimulus applied between 300 and 700 msec after the first. These results suggest that nigral GABA-receptive neurons may be directly involved in the regulation of EtOH withdrawal seizure activity, or indirectly through their effects on other subcortical sites.

283.13 SENSITIVITY TO ANTICONVULSANT EFFECTS OF HYPNOTICS IN MICE BRED TO BE WITHDRAWAL-SEIZURE-PRONE (WSP) AND -RESISTANT (WSR) AFTER ETHANOL PHYSICAL DEPENDENCE. J.C. Crabbe, A. Kosobud*, E.R. Young*, B.R. Tam*, and J.D. McSwigan*. VA Medical Center and Depts. of Medical Psychology and Pharmacology, Sch. of Med., Oregon Health Sciences Univ., Portland, OR 97207 USA.

We are selectively breeding mice to be resistant (WSR) or prone (WSP) to develop withdrawal seizures after chronic treatment with ethanol vapor. After twelve generations of selection, the WSP line develops approximately 10-fold more severe withdrawal seizures than the WSR line. We have previously shown that the WSP and WSR lines have virtually identical thresholds for seizures induced by a variety of drugs and treatments. Ethanol (1.5 g/kg) was shown to be a generally effective anticonvulsant agent in both lines, and the WSR line is markedly more sensitive than the WSP line to the anticonvulsant effects of ethanol against seizures induced by electroconvulsive shock (ECS). The WSR line is slightly more sensitive to ethanol's anticonvulsant effects against flurothyl and strychnine, but the lines do not differ in sensitivity to ethanol against bicuculline, picrotoxin, or pentylenetetrazol seizures.

We compared the WSP and WSR lines one hour after treatment with methanol (2 g/kg), ethanol (1.5 g/kg), n-propanol (0.75 g/kg), or t-butanol (0.37 g/kg) by establishing the Convulsive $Amperage_{50}$ (CA_{50}) for tonic hindlimb extensor seizures induced by transcorneal ECS (200 msec, varying mAmp, using the up-and-down method of Dixon and Mood). All treatments elevated seizure thresholds, and the WSR mice were more sensitive than the WSP mice to these effects in all cases. The ratio (WSR:WSP) of CD_{50} after treatment was approximately 2 for all alcohols tested. Thus, there did not appear to be a sensitivity difference between the lines as a function of chain length of alcohol tested. When sodium pentobarbital (45 mg/kg) was tested, the opposite result was found. WSP mice were approximately 1.6 times more sensitive than WSR mice. These data suggest that differences in sensitivity to the anticonvulsant effects of hypnotics are controlled by different sets of genes. Further, they indicate no simple relationship between genetically determined sensitivity to develop ethanol withdrawal seizures and sensitivity to hypnotics.

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283.14 EXPERIMENTALLY INDUCED GLUCOSE INTOLERANCE INCREASES ORAL ETHANOL INTAKE IN RATS. K. A. Zito and D. C. S. Roberts, Carleton University, Ottawa, Canada, K1S 5B6.

A number of studies have shown a relationship between glucose tolerance and ethanol intake. Although there seems little doubt that biochemical disturbances may be consequential to chronic ethanol intake, the converse of this may also hold. That is, an altered neurochemical or metabolic status such as glucose intolerance, may influence the amount of ethanol consumed. While evidence exists in animals that a genetically-linked glucose intolerance can affect alcohol intake, we sought to answer whether a dietary-induced glucose intolerance might also affect alcohol consumption. In the present study we have used a relatively simple procedure to induce glucose intolerance to test whether this condition is sufficient to produce an increase in chronic ethanol intake in rats.

Subjects were assigned to equal groups (n=12) where they were given access to one of four solutions: peppermint-flavored sucrose (40%), peppermint-flavored saccharin (0.1%), peppermint in water (0.1%) or water alone, presented three times a week, for a period of 11 weeks. After 12 weeks all animals were subjected to an oral glucose tolerance test which revealed that the chronically prepared sucrose animals had become glucose intolerant. At the start of week 13 all animals were given access to a 6% ethanol solution flavored with peppermint in place of the previous solutions, for a period of 11 weeks.

Sucrose animals displayed an immediate preference for ethanol and consumed approximately three times more ethanol than the remaining groups. The results of this study indicate that rats that are made glucose intolerant by longterm access to a high concentratiion of sucrose, when given the opportunity, will subsequently drink more ethanol than control animals.

283.15 CHRONIC ETHANOL AND (Na^+,K^+)-ATPase: EVIDENCE FOR A MEMBRANE ADAPTATION. A.C. Swann. Dept. of Psychiatry, University of Texas Medical School, Houston, TX77025.

Our previous work has shown that the conformation of (Na^+,K^+)-ATPase depends on membrane fluidity (Arch. Biochem. Biophys. 221:148) and that effects of ethanol added in vitro are consistent with those of increased membrane fluidity (J. Biol. Chem. 258:11780). These effects include reduced affinity for K^+ at its allosteric site at all temperatures, increased ΔH and ΔS for K^+ binding, and reduced ΔH and ΔS for the principal conformational transition of the enzyme. We have now examined effects of chronic ethanol feeding. Rats were pair-fed with Lieber-DiCarli diets containing 5% ethanol or isocaloric replacement. Ethanol-treated rats gained weight satisfactorily and were tolerant to ethanol (sliding angle test). They were killed after four weeks of ethanol treatment (plasma ethanol = .196 $\pm$ .057 (S.D.; n=8)) and cerebral cortex microsomes were prepared. K^+-p-nitrophenylphosphatase activity associated with (Na^+,K^+)-ATPase from ethanol-fed rats was less sensitive to inhibition by ethanol than was that from controls (either liquid diet or chow-fed), especially at low K^+ and high temperature (optimal conditions for ethanol inhibition). Apparent affinity for K^+ was increased at all temperatures. ΔH and ΔS for K^+ binding were decreased in ethanol-fed rats compared to controls, while those parameters for the conformational transition were increased. These changes were opposite to the effects of ethanol when added in vitro. These data suggest that changes in the cell membrane during ethanol treatment render (Na^+,K^+)-ATPase less susceptible to inhibition by ethanol.

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283.16 A POSSIBLE MODEL FOR ETHANOL'S EFFECT ON SELECTIVE ATTENTION: DISRUPTION OF SENSORY GATING IN RAT CORTEX. J.K. Chapin, S.M. Sorensen, and D.J. Woodward. U. of Texas Health Science Center., Dallas, Texas, 75235.

We have developed a model system for studying alcohol effects on sensorimotor and selective sensory processing in the cortex. This involves recording single units in the forelimb and paw areas of the somatosensory (SI) and motor (MI) cortices of awake rats. A phasic, selective inhibition (or "gating") of sensory transmission from the forepaw to single SI cells was shown to occur over the forelimb step cycle during treadmill locomotion. This gating may function to filter sensory information coming from the forepaw and to route it to different classes of cortical cells according to the timing of the movement. Specifically, some cells only responded to forepaw stimulation around the time of footfall. Others responded only during swing phase, and thus may have been primed to selectively respond when obstacles were unexpectedly encountered. Our hypothesis is that the phasic inhibition subserving this gating may be caused in part by axonal projections from nearby motor (MI) cortical cells. We have shown that a large percentage of such MI cells discharge strongly during the active forelimb reaching movement which just preceeds footfall during stepping. Inhibitory or facilitory synaptic influences, coursing from the MI to the SI just before footfall, could subserve the observed sensory gating phenomena. The dose-dependent effect of alcohol on this model circuit was tested by recording single neurons in the SI or MI cortices and measuring their physiological response properties before and after ethanol injection (20% ETOH w/v in saline, I.P. or I.G.). Moderate ethanol doses (as low as 0.3g/Kg) caused a marked reduction in the overall level of inhibitory gating of sensory transmission to SI cortical cells during movement. Since low dose ethanol had minimal effect on the neuronal sensory responses themselves, it actually increased the sensory input to the cortex during movement, but reduced its selectivity. Neurons were also recorded in the MI cortex. Those which fired in correlation with active forelimb movement were classified as "Active Joint" (AJ). Those which also responded to passive movement of the same limb were "Passive Joint" (PJ). Moderate ethanol doses (as low as 0.5g/Kg) strongly reduced or abolished the motor correlated discharges of the AJ's, but not the PJ's. We thus conclude that ethanol may disrupt sensory gating partly by altering "gating bias signals" emanating from the MI cortex. Supported by grants NS18041, AA0390 and the Biological Humanics Foundation.

283.17 EFFECT OF CNS NORADRENERGIC LESION BY DSP4 ON ETHANOL SLEEPTIME IN LS AND SS MICE. K. Spuhler*, G. Gerhardt and M. Palmer (SPON: J. Masserano). Alcohol Research Center, Department of Pharmacology, University of Colorado School of Medicine, Denver, CO 80262.

To test the hypothesis that central noradrenergic (NE) pathways might be associated with genetic differences in acute ethanol sensitivity in LS (long-sleep) and SS (short-sleep) selected lines, we used N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4) to reduce central NE levels. DSP4 has been found to be a relatively selective neurotoxin for noradrenergic neurons; however, serotonin- (5-HT) containing neurons are somewhat affected. Dopamine- (DA) containing neurons are spared.

DSP4 (50 mg/kg body weight) or saline was administered in a single dose IP to groups of LS and SS male mice from the 33rd and 34th generations of breeding at 42 days of age in two replicate experiments. These animals were tested for "sleeptime" at 60 days of age. SS mice were administered an ethanol dose IP of 4.5 g/kg body weight and LS mice, a 3.5 g/kg ethanol dose IP (from a 20%, w/v, solution in 0.9% saline) for the sleeptime test. In a third experiment DSP4 or saline was administered twice, at 42 days and at 60 days of age. These animals were tested for "sleeptime" at 75 days of age. Four to six days after recovery from the sleeptime test animals were sacrificed and their brains dissected, quick-frozen with dry ice and weighed. Tissue content of NE, DA and 5-HT in the brainstem, cerebellum and striatum was quantitated by HPLC with electrochemical detection.

For DSP4-treated and control mice, the % depletion from control levels were: 1) NE - 48% in SS, 55% in LS in brainstem; and 78% in SS, 70% in LS in cerebellum; 2) 5-HT - 25% in SS, 39% in LS in brainstem; and 20% in SS, 12% in LS in cerebellum; and 3) DA - 11% in SS, 17% in LS in striatum. For the SS line the duration of loss of righting reflex (min) was 28.4 $\pm$ 2.7 (N=16) in the DSP4-treated group and 28.2 $\pm$ 3.2 (N=13) in the control group. The LS line sleeptime was 145.0 $\pm$ 7.7 (N=12) in the DSP4-treated group and 132.4 $\pm$ 5.4 (N=18) in the control group. Thus, there was no change in sleeptime in the SS due to CNS noradrenergic depletion, and only a slight nonsignificant increase in sleeptime of DSP4-treated LS mice compared to their respective controls. These results suggest that central noradrenergic pathways do not play a major role in the genetically-selected behavioral sensitivity differences to ethanol observed in these two mouse lines.

283.18 ELECTROPHYSIOLOGICAL AND NEUROENDOCRINE MEASURES IN MALE ALCOHOLICS AND THEIR SONS. S.C. Whipple*, C. Berka*, R.E. Poland* and E.P. Noble* (SPON: J.T. Cummins). Alcohol Research Center, Neuropsychiatric Institute, University of California, Los Angeles, CA 90024.

Evidence suggests that genetic factors play an important role in the development of alcoholism in sons of male alcoholics. The identification of biological markers, therefore, could assist in the prevention and early diagnosis of this disease.

The present research was designed to assess EEG spectral content and multiple neuroendocrine parameters in male recovered alcoholics and their sons. Father and son pairs were studied in relation to age-matched controls in a five-hour testing session during which subjects engaged in selective attention and continuous performance tasks. The goal of the study was to induce changes in the subjects' behavioral state which would allow an examination of the relationship between neuroendocrine and electrophysiological function during stress, vigilance and mental effort.

The EEG was sampled from 11 electrode sites for one min in an eyes open condition and one min in an eyes closed condition. Blood samples were obtained concurrently with the EEG spectral samples at 10 points during the five-hour session. Hormones which were measured were cortisol, ACTH, prolactin, testosterone, and growth hormone. Following each EEG data acquisition epoch, power spectra for both conditions were computed via a fast Fourier transform. The average power in the alpha (8-12 HZ), slow beta (12-20 Hz), and fast beta (20-30 Hz) frequency bands was obtained. For each subject separate stepwise linear regression analyses were performed for each condition (eyes open and eyes closed) and for each EEG frequency band. The frequency bands were used as the dependent variables and the hormone measures as the predictor variables.

Each subject showed a characteristic pattern of correlations among responses. Complex interrelationships emerged between endocrine and EEG measures in all groups tested. (This study was supported by The Seaver Institute.)

283.19 AN EXPERIMENTAL MODEL OF SELF-INTOXICATION IN C57 MICE.
R. Thomas Gentry, Rockefeller University, 1230 York Ave., New York, NY 10021.

C57 mice offered unlimited free access to water and a 10 % solution of ethanol have relatively low mean plasma concentrations of ethanol (25 ± 2 mg/dl) despite a daily rate of intake (8.8 ± 0.2 g/kg) several fold greater than human alcoholics.

Mice treated with the alcohol dehydrogenase inhibitor, 4-methylpyrazole (4MP) delivered by osmotic minipumps, metabolized ethanol at rates comparable to humans, and representing a daily capacity to eliminate approximately 3 g/kg. Mice treated with 4MP and offered ad libitum ethanol, food and water, decreased their intake of ethanol to 2.9 g/kg per day, but exhibited severe intoxication. Mean plasma ethanol concentrations at midnight were 254 + 31 mg/dl, ten-fold greater than controls receiving chronic infusions of saline with no overlap between groups.

A redistribution of ethanol intake into the 12 hours of the light period (48 % of the daily total, compared to 23 % in controls) contributed to the maintenance of plasma concentrations in excess of 150 mg/dl throughout the 24-hour day. Some subjects in the experimental group exhibited severe toxicity as indicated by a critical loss in body weight during the second week of treatment. After removal of the ethanol bottle three of these mice recovered and one died. This toxicity, however, was self-induced since one of the necessary components (ethanol) was voluntarily self-administered. Other mice given the same dose of 4MP by chronic infusion with only water to drink exhibited no weight loss.

The results suggest that mice with a chronic reduction in their capacity to eliminate ethanol fail to adjust their voluntary consumption of ethanol to avoid severe intoxication and toxicity.

This work has been supported by a grant form the John L. and Helen Kellogg Foundation to Dr. Vincent Dole.

ALCOHOL AND BARBITURATES III

284.1 ETHANOL-DEPENDENCE AND CALCIUM RELEASE IN ERYTHROCYTE GHOSTS
Usha Pande*, Harish C.Pant & Edward Majchrowicz*. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

In previous studies (Soc. Neurosc. Abs. 7, 1235, 1983) we have shown that in vitro addition of ethanol affects the intracellular calcium in synaptosomes isolated from rat brain. In the present report, we studied the in vitro and in vivo effects of ethanol on calcium metabolism in erythrocyte ghosts derived from control and ethanol-dependent rats. Tolerance and physical dependence upon ethanol were induced in male Sprague-Dawley rats (250-350g) as previously described (Majchrowicz, E. Psychopharmacologia, 43: 245, 1975). Blood was collected from five groups: (1) pair fed controls, (2) dependent-intoxicated (prodromal phase), (3) dependent-withdrawing (ethanol withdrawal syndrome), (4) 4 hr after an acute single dose of ethanol (6g/kg), and (5) 20 hr after an acute single dose of ethanol. Erythrocyte ghosts were isolated according to Hanahan & Ekholm (Methods in Enzymology 31: 168, 1974). The effect of ethanol treatment on calcium metabolism was studied employing the calcium indicator dye Arsenazo III (Az). Resealed ghosts were resuspended in isotonic medium (pH 7.32) containing 20mM Tris-HCl, 132mM NaCl, 5mM KCl, 1mM EGTA, 0.85mM $CaCl_2$ and 9uM Az. Absorbance (650-750nm) of Ca-Az complex (ΔA) was measured in a suspension of ghosts (30ug/mg protein) in isotonic medium. In the ghosts isolated from dependent-intoxicated rats, ΔA increased by 110±2% (control=100%) and in the case of withdrawal the increase was 140±5%. Absorbance of group (4) ghosts increased 135±5%, whereas that of group (5) was similar to control. In vitro addition of 100mM ethanol to control samples caused an increase in ΔA to 160±6%, dependent-intoxicated group exhibited a decrease from 110±2% to 100±6%. However, in the withdrawing group (ΔA=140±5%), in vitro addition of 100 mM ethanol caused no change in ΔA. Under the present experimental conditions, we found that Az dye neither binds to, nor is permeable to erythrocyte ghost membranes. Thus, the measured changes in ΔA are presumed to reflect a release of calcium from the ghosts. These observations suggest that induction of physical dependence upon ethanol is associated with a suppression of ethanol-induced calcium release.

284.2 FETAL EXPOSURE TO ETHANOL AFFECTS SENSITIVITY TO MORPHINE BUT NOT BRAIN OPIATE RECEPTOR BINDING IN RATS.
L.R. Nelson, A.N. Taylor, B.J. Branch*, J.C. Liebeskind, and J. W. Lewis. Depts. of Psychology and Anatomy, Lab. of Neuroendocrinology, Brain Research Inst., UCLA; West LA VA Med. Ctr., Brentwood, Div., Los Angeles, CA 90024, and Mental Health Research Inst., Univ. of Michigan, Ann Arbor, MI 48109.

Fetal ethanol exposure (FEE) can produce long-lasting behavioral and hormonal alterations in rats. We have shown that FEE results in enhanced opioid-mediated, stress-induced analgesia. In addition, we have shown that FEE results in enhanced responding to morphine on three measures: analgesia, pituitary-adrenal activation, and hypothermia. In light of these findings, the present study was conducted to assess the possibility that FEE produces alterations in brain opiate receptors.

Subjects were female offspring of Sprague-Dawley dams fed either a 5% w/v ethanol-containing casein-supplemented liquid diet (BioServ) ad lib (FEE), pairfed an isocaloric liquid diet without ethanol (PF), or given lab chow and water ad lib (N), from gestation day 8 to birth. At 140 days of age rats were sacrificed and the brains were removed and dissected on ice into the following 7 regions: frontal cortex, striatum, thalamus, hypothalamus, midbrain, hippocampus, and pons/medulla. Brain areas from 7-8 rats in each prenatal treatment group were pooled, homogenized in TRIS buffer containing 5% DMSO and frozen until assayed. Two populations of opiate receptors were examined: mu receptors using 3H-DAGO (D-Ala-NePhe-Glyol-enkephalin) and delta receptors using 3H-DADL (D-Ala-D-Leu-enkephalin). Binding assays were carried out according to the procedures of Akil et al. (Eur J Pharm 64:1-8, 1980). Data were analyzed by Scatchard plots.

There were no differences among the groups in binding characteristics (Kd and Bmax) of 3H-DAGO or 3H-DADL in the cortex, striatum, thalamus, midbrain, hippocampus, or pons/medulla. The only brain region with pronounced differences between groups was the hypothalamus. In this region, there was decreased mu and delta binding in both FEE and PF rats as compared to N rats. Thus, it appears that prenatal nutritional factors affect opiate receptors in the hypothalamus, while sensitivity to morphine is augmented selectively by prenatal ethanol exposure. (Supported by VA Medical Research Service, NIH grant NS07628, and a gift from the Brotman Foundation).

284.3 **ACETYLCHOLINE TURNOVER IN SPRAGUE-DAWLEY RATS: THE EFFECT OF A LOW DOSE OF ETHANOL.** A. Kochhar* and C.K. Erickson. College of Pharmacy, Univ. of Texas, Austin, TX. 78712.

Hypnotic doses of ethanol alter the turnover rates of many central neurotransmitters. This nonspecific effect of ethanol may be associated with its membrane disordering properties. Low doses of ethanol may produce more discrete actions on certain neurotransmitter systems. The present study examines the effect of a low dose of ethanol on the central cholinergic system. Female Sprague Dawley rats received 1.5 g/kg ethanol (10% w/v) or saline intraperitoneally. Animals were sacrificed by microwave irradiation 15 or 30 minutes after injection. D_4 choline was pulse injected into the tail vein 1 minute before sacrifice. Endogenous and deuterium-labeled variants of acetylcholine (ACh) and choline (Ch) were analyzed by gas chromatography/mass spectrometry. Acetylcholine turnover rate (ACh TO_R) was calculated in the cortex, hippocampus, midbrain and striatum. ACh TO_R decreased in all brain areas 15 minutes after ethanol administration. The cortex and hippocampus showed the greatest depression in TO_R. Cortical and hippocampal ACh TO_R were further depressed 30 minutes after ethanol. ACh and Ch concentrations decreased only in the hippocampus 15 minutes after ethanol; however, other brain area concentrations decreased 30 minutes after ethanol. The results show that a low dose of ethanol reduces cholinergic activity in several brain regions. Some brain areas appear to be more sensitive to ethanol than others. (Supported by AA04114.)

284.4 ETHANOL CHRONIC CONSUMPTION ALTERS STRIATAL OPIATE FUNCTION: RELATIONSHIP WITH SALSOLINOL EFFECTS - L. Lucchi*, R.A. Rius*, S. Govoni, M. Trabucchi[a]. [a]Chair of Toxicology, II University of Rome and Institute of Pharmacology and Pharmacognosy, University of Milan, Milano 20129, Italy.

Chronic ethanol treatment produces supersensitivity of striatal μ-opiate receptor sites labelled by ^{3}H-Etorphine (higher Bmax) and ^{3}H-DADLE (higher affinity). These results have been correlated to the marked decrease of K^+-stimulated met-enkephalin immunoreactive material (ME-IR) release detected in striatal slices from rats (male Sprague-Dawley) chronically exposed to ethanol (6% aqueous drinking solution for 25 days). On the contrary, a lower affinity of striatal μ-opiate receptors labelled by ^{3}H-DHM was evidenced after the same ethanol regimen. The different sensitivity of the two classes of striatal opiate receptor to ethanol may be a consequence of selective effects of alcohol on enkephalinergic system. In fact, it has been hypothesized that the "down regulation" of μ-receptors takes place because of an enhanced formation of endogenous substances, such as salsolinol, derived from ethanol-metabolism preferentially interacting with μ-receptors. The purpose of the present work was to compare the effects of chronic salsolinol treatment (40 mg/kg i.p., 21 days) on ME-IR release and opiate receptor function with the ethanol-induced changes. The results indicate that "in vitro" salsolinol does not displace ^{3}H-DADLE binding to striatal membranes but interacts with sites labelled by ^{3}H-DHM. In "in vivo" studies chronic salsolinol treatment did not affect ^{3}H-DADLE and ^{3}H-Etorphine binding to striatal membranes. On the contrary, a decreased affinity of ^{3}H-DHM was observed in striata from rat chronically treated with salsolinol. In addition, the effect of salsolinol on ME-IR release differed from that of ethanol treated rats.

284.5 NEONATAL ETHANOL EXPOSURE TO RAT PUPS AND THE RESULTANT ALTERATIONS OF CEREBELLAR H_1-HISTAMINE RECEPTOR KINETICS. K. E. Light, College of Pharmacy and Division of Interdisciplinary Toxicology, College of Medicine, University of Arkansas for Medical Sciences, 4301 West Markham Little Rock, Arkansas 72205

We have been investigating the alterations of brain neurotransmitter systems induced by postnatal ethanol exposure to Sprague-Dawley rat pups. Male pups were equipped with an intragastric-cannula on postnatal day 4 (PN4) and artifically-reared (AR) using a diet similar in composition to rats' milk. To the diet was added either ethanol 3-5% v/v (~ 7-12g/kg/day) or isocaloric dextrose. Pups were exposed for four days (PN4-8) and then either sacrificed or returned to lactating dams and reared until PN20 without further ethanol exposure. H_1-histamine receptor kinetics were determined in a cerebellar (CB) membrane homogenate using (^{3}H)-pyrilamine with 5μM triprolidine to determine non-specific binding. Binding kinetics were also determined in cerebellar membranes from mother-raised control rat pups at PN8 and PN20. AR pups showed decreased CB wet weight at PN8. In addition, the P_2-membrane protein content was significantly increased. Ethanol had no consistant effect on CB weight or P_2-protein content either at PN8 or PN20. In control rat pups the ontogeny of cerebellar H_1-histamine receptors, from PN8 to PN20, involves a five-fold decrease in affinity and a five-fold increase in number. Exposure to 4% ethanol (PN4-8) caused an increase in affinity (3x) and a decrease in receptor number (2x) when measured at PN8. Both effects were reversed when similarily treated pups were returned to mothers and reared until PN20. Exposure to 5% ethanol (PN4-8), however, results in a complete disruption of the normal ontogeny of cerebellar H_1-receptors even though pups were returned to mothers and reared until PN20 without further ethanol exposure. In these animals cerebellar H_1-receptors showed binding kinetics at PN20 identical to normal PN8 rat pups. We conclude that short-term ethanol exposure can cause severe, long-lasting alterations in cerebellar H_1-receptor kinetics.
(Supported by NSF grant ISP 8011447 and the UAMS Foundation Fund).

284.6 INTERACTION OF ETHANOL AND NALOXONE ON PROLACTIN SECRETION IN RAT. V. Rettori*, A. Seilicovich*, B. Duvilanski*, M. Rubio* and V. Muñoz-Maines* (SPON: L. Hersh). Centro de Invest. en Reproduccion, Fac. de Medicina, UBA, and Inst. de Investigaciones Farmacologicas, CONICET, Buenos Aires, Argentina.

Previous studies have shown that exogenous opioids can elevate serum prolactin (Prl) levels. Recently, we have found that the acute administration of ethanol (ETOH) can cause an increase in serum Prl without affecting pituitary Prl content. Others have found that ETOH administration causes an increase in endogenous opioids in the hypothalamus. In order to study the possible mediation by endogenous opioids of the ETOH-induced elevation of serum Prl levels, we administered Naloxone (Nal) following ETOH pretreatment and measured concentrations of serum and pituitary Prl and hypothalamic dopamine (DA). A total of 32 Wistar adult male rats were injected i.p. with saline (Sal or ETOH [5g/kg]). After 45 min. either Sal or Nal (0.4 mg/rat) was injected i.p. for 15 min. prior to decapitation. Pituitary and serum Prl was measured by RIA, and DA by HPLC. Duncan's multiple-range test was used for statistical analysis. Serum Prl was lower in the group treated with Nal (Sal-Nal: 15.84±2.05 ng/ml) as compared to values in the saline-treated group (Sal-Sal: 24.36±4.58). ETOH increased serum Prl levels (ETOH-Sal: 363.33±58.68) but this increase was significantly higher than in the group that received Nal (ETOH-Nal: 165.50±35.35). There were no significant differences in the concentration of pituitary Prl between any of the four treatment groups. DA concentration in the hypothalamus of Nal-treated rats was lower (Sal-Nal: 0.25±0.02 ug/g) than in the saline group (Sal-Sal: 0.32±0.02). ETOH increased DA concentration (ETOH-Sal: 0.43±0.02) but Nal reversed this increase (ETOH-Nal: 0.33 ± 0.01). These results suggest that the serum Prl increase induced by ETOH could be mediated at least in part by endogenous opioids through a dopaminergic mechanism.

284.7 ENHANCEMENT OF GABAERGIC INHIBITION BY BARBITURATES AND RELATED DEPRESSANT DRUGS IN RAT HIPPOCAMPAL SLICES. T.V. Dunwiddie and T.S. Worth*. Veterans Administration Medical Research Service and Department of Pharmacology, Univ. Colorado Health Sci. Center, Denver, CO 80262.

We have characterized the effects of convulsant and nonconvulsant barbiturates on electrophysiological responses in the rat in vitro hippocampal slice preparation. Several barbiturate analogs were found to markedly enhance the duration of the recurrent feedback GABAergic inhibition elicited by antidromic stimulation of the axons of CA1 pyramidal neurons. This effect was observed with DMBB (5-ethyl,5-[1,3-dimethylbutyl] barbituric acid, pentobarbital and mephobarbital; concentrations of these drugs required to double the duration of inhibition were 10 uM, 20 uM, and 60 uM respectively. The maximal responses to these drugs did not differ significantly, and represented a 10-20X increase in the duration of inhibition following a test response. Phenobarbital was completely inactive in increasing the duration of inhibition at concentrations up to 300 uM. Other non-barbiturate drugs such as etomidate and etazolate were found to elicit similar changes in GABAergic inhibition.

In addition to their effects on inhibitory responses, barbiturates were also found to affect responses to stimulation of excitatory afferents to CA1 pyramidal neurons. In low concentrations, some barbiturates such as pentobarbital and DMBB increased the evoked population spike response, primarily by increasing the amplitude of the EPSP. However, at high concentrations, many of the barbiturates had anesthetic effects which were quite similar to those observed with local anesthetics such as cocaine and lidocaine. These direct effects did not appear to be correlated with effects on GABAergic inhibition.

Thus, the barbiturates which we have examined have multiple effects in the hippocampus, and the dose ranges required to elicit these responses show significant overlap. The enhancement of GABAergic inhibition appears to be highly correlated with the potencies of these barbiturates in enhancing the binding of benzodiazepines and GABA to binding sites in brain membranes (Olsen and Snowman, J. Neuroci. 2:1812, 1982; Leeb-Lundberg et al., PNAS 77:7468, 1980), while the excitatory responses appear to be related to the convulsant properties of some barbiturates.

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284.8 THE CEREBELLAR cGMP SYSTEM AS A NEUROCHEMICAL INDEX OF BARBITAL TOLERANCE AND WITHDRAWAL. S.J. Lane* and W.W. Morgan. Department of Cellular and Structural Biology, The Univ. of Tex. Health Sci. Ctr. at San Antonio, San Antonio, TX 78284.

That chronic barbiturate use results in the development of behavioral tolerance is well established. Subsequent abrupt withdrawal of these drugs precipitates a very severe abstinence syndrome. We recently demonstrated that neurochemical tolerance of the cerebellar cGMP (Cb cGMP) system could be induced in animals exposed to a 6 wk barbital feeding regimen. As a continuation of this work we have investigated the time course of both tolerance development and withdrawal induced alterations in the Cb cGMP system. To investigate the time course of tolerance development groups of female ovariectomized rats were begun on the feeding regimen at varying times and thus, on the day of sacrifice, rats had received barbital for 37, 33, 30, 19, 16, 5 or 2 days (d). Rats were sacrificed by focused microwave irradiation and cerebellae and cerebral cortices dissected and frozen for assay. In two subsequent experiments all animals went through the entire feeding regimen. Then, at 0 hrs barbital was withdrawn and animals were sacrificed at 0, 2, 4, 6, 8, 12, 24, 36, 48 and 72 hrs post-withdrawal (p-w). Brain parts were collected as above. During the early stages of chronic barbital administration Cb cGMP was significantly reduced (242±17 vs controls: 647±43 pmol/gm at 2 d) but gradually returned almost to control levels by 37 d (497±52 pmol/gm). Cerebral cortical cGMP (C cGMP) was also depressed but less profoundly (control: 216±18; 2 d: 113±7; 30 d: 177±35 pmol/gm). Abrupt withdrawal of barbital brought about a very rapid, highly significant elevation of Cb cGMP (4 hr p-w: 3632±651 vs control: 1173±147 pmol/gm) which occurred just after the onset of weight loss but in advance of locomotor depression and onset of spontaneous seizures. C cGMP failed to change during withdrawal. At 72 hr p-w Cb CGMP had only begun to return towards control values and weight loss continued to be highly significant. Other indices had normalized by 48 hr p-w.

These results clearly demonstrate that Cb cGMP is a highly useful neurochemical index of barbital induced CNS changes. Alterations of Cb cGMP probably reflect a barbiturate influence on one or more transmitter system(s) involved in cGMP regulation. Precisely which system(s) are involved awaits further investigation. Supported by DA00755 and DA00083.

284.9 THE EFFECTS OF HYPERBARIC PRESSURE AND ANESTHESIA ON EVOKED POTENTIALS IN THE TURTLE CORTEX. D. L. Tauck and J. J. Kendig. Dept. of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305.

Most previous studies of anesthetic-pressure interactions on isolated neuronal tissue have employed either peripheral structures or invertebrate ganglia. The relevance of the results to the mechanism of anesthesia in the vertebrate central nervous system remains to be established. A vertebrate brain preparation which appears advantageous for studies of anesthesia and anesthetic-pressure interactions is the cerebral cortex of the turtle, Pseudemys scripta. The isolated turtle cortex has proved more robust under the requirements imposed by the pressure chamber than either guinea pig or rat hippocampal slices. The cortex, which consists of a single layer of nerve cells, is removed from the brain and submerged in a temperature-controlled chamber perfused with oxygenated saline buffered with 10 mM HEPES. Compression was achieved by admitting helium from a commercial high pressure cylinder at a rate slow enough to prevent temperature fluctuations larger than 1°C. Similarly, the rate of decompression was also controlled to maintain nearly constant temperature in the recording chamber. Evoked field potentials monitored with extracellular electrodes reveal an early, directly evoked negativity followed by a larger, slower, Ca^{2+}-dependent response. Inhibition can be detected by paired pulses. Preliminary experiments show that pentobarbital, 1-3 x 10^{-4}M reversibly depresses the Ca^{2+}-dependent response. These concentrations are comparable to those effective on other vertebrate CNS preparations, and within the anesthetic concentration range for this agent. Hyperbaric pressure up to 150 atmospheres had no effect on the directly evoked potential, but reversibly depressed the Ca^{2+}-dependent response. Pressure up to 100 atmospheres does not reverse pentobarbital-induced depression of the Ca^{2+}-dependent response, a finding consistent with results from other neural preparations. The reversibility of anesthetic and pressure effects even after some hours of exposure attests to the validity of this preparation for such studies.

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284.10 EFFECT OF ETHANOL ON THE ENZYMES RESPONSIBLE FOR LIVER GAMMA-HYDROXYBUTYRIC ACID METABOLISM. O.C. Snead and F. Poldrugo. Department of Pharmacology, Pediatrics and The Neuroscience Program, University of Alabama in Birmingham School of Medicine, Birmingham, Alabama 35233.

Ethanol has been shown to increase endogenous gamma-hydroxybutyric acid concentrations in liver (Poldrugo et al, Neurosci. Abstr. 8,651,1982); however, the mechanism for this ethanol-induced increase in liver concentration of γ-hydroxybutyrate GHB is not clear since most of the work done to date concerning the metabolism of GHB has been done primarily in rat brain rather than liver.

The object of the present study was therefore to investigate possible sites of ethanol interactions with GHB metabolism in vitro in rat liver homogenate.

We measured the effect of ethanol and pyrazole on the derivation of GHB from succinic semialdehyde, the conversion of succinic semialdehyde to GHB, and the formation of GHB from another possible precursor, 1,4-butanediol.

Pyrazole blocked and ethanol competitively inhibited the NADH dependent enzyme responsible for the conversion of succinic semialdehyde to GHB and the NAD dependent enzyme responsible for the conversion of GHB to succinic semialdehyde. Moreover, ethanol competitively inhibited the NAD dependent conversion of 1,4-butanediol to GHB (Maxwell et al, Biochem. Pharmacol. 21.1521,1972). Horse liver alcohol dehydrogenase was able to convert GHB to succinic semialdehyde.

These data indicate multiple sites of ethanol interaction with GHB metabolism in liver. Our results suggest that the increase in endogenous liver GHB seen after ethanol is related to competition between ethanol and GHB for liver alcohol dehydrogenase.

284.11 POSSIBLE ROLE OF ENDOGENOUS 1,4 BUTANEDIOL IN THE MECHANISM OF ACTION OF ETHANOL. F. Poldrugo, O.C. Snead and S. Barker. Department of Pharmacology, Pediatrics, The Neuroscience Program, and The GC/MS Center, University of Alabama in Birmingham School of Medicine, Birmingham, Alabama 35233.

1,4-butanediol has been reported to potentiate the behavioral effects of ethanol in rats and an in vivo competition between the two substances for alcohol dehydrogenase has been postulated (Poldrugo et al, Neurosci. Abstr. 9,1234,1983).

The object of these experiments was twofold: first to determine which of these compounds is responsible for behavioral changes observed when both are given together and secondly whether they compete for alcohol dehydrogenase in brain as well as in liver.

1,4-butanediol was administered concomitantly with ethanol to rats and brain and liver concentrations of 1,4-butanediol measured. In addition, the conversion of 1,4-butanediol to its major metabolite, γ-hydroxybutyric acid, a reaction mediated by alcohol dehydrogenase, was monitored by measuring gamma-hydroxybutyric acid. Blood ethanol levels were also measured.

A dose of 1 g/Kg 1,4 butanediol increased 1,4-butanediol levels in brain and liver, but did not increase blood ethanol levels. Ethanol lowered the conversion of 1,4-butanediol to GHB and increased the concentration of 1,4-butanediol in brain and liver after exogenous administration.

These data suggest that ethanol acts on 1,4-butanediol degradation in both brain and liver through competition with alcohol dehydrogenase.

The results may be relavent in the mechanism of action of ethanol considering that endogenous 1,4-butanediol is normally present in brain and liver (Barker et al, Neurosci. Abstr. 9,1105,1983) and is particularly toxic in animals and humans (Hinrichs et al, Pharmazie 3,110,1948).

284.12 THE EFFECT OF FETAL EXPOSURE TO ETHANOL ON LUTEINIZING HORMONE-RELEASING HORMONE AND LUTEINIZING HORMONE IN PREPUBERTAL AND POSTPUBERTAL RATS. D.L. Morris*, N.H. McArthur and P.G. Harms*. Departments of Veterinary Anatomy and Animal Science, Texas A&M University, College Station, TX 77843.

Previous investigations in our laboratory have demonstrated a significant increase in hypothalamic luteinizing hormone-releasing hormone (LHRH) and decrease in serum luteinizing hormone (LH) following exposure of adult male rats to ethanol (ETOH).

The purpose of this study was to determine the effects of fetal and postpartum exposure to ETOH on hypothalamic LHRH and serum LH in prepubertal and early postpubertal male and female rats. Thirty-six 100-day-old female rats were bred and exposed to ETOH. Control animals were fed an isocaloric Bio-Serve liquid diet. ETOH-exposed animals received a Bio-Serve 30% ETOH-derived caloric liquid diet. Treatment groups were exposed during gestation only, during lactation only, or during gestation and lactation. The control and ETOH exposed offspring were weaned on day 20 and given rat lab chow and water ad libitum. Offspring were decapitated at 20, 30 and 40 days of age and trunk blood was taken in EDTA tubes and centrifuged. The serum was stored at -20°C. Following re-lyophilization, the basal hypothalami were extracted with HCL and radioimmunoassayed for LHRH content.

The hypothalamic LHRH content of male rats exposed to ETOH during gestation and/or during suckling was significantly lower than the controls at 20, 30 and 40 days of age. The hypothalamic LHRH content of female rats exposed to ETOH during gestation and/or suckling was significantly lower than the controls at 30 and 40 days of age.

No significant differences in plasma LH were seen between the control groups and the ETOH treatment groups of either male or female rats at the various ages.

These data demonstrate that fetal and/or lactation exposure to ETOH significantly reduced hypothalamic LHRH content. This suggests a direct effect of ETOH on the synthesis and/or storage of LHRH by hypothalamic LHRH neurons in the fetal and prepubertal rat, this effect of ETOH appears to be different from that in the adult rat hypothalamus.

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284.13 ETHANOL-INDUCED INHIBITION OF SPONTANEOUS FIRING OF LOCUS COERULEUS NEURONS IS ASSOCIATED WITH AN ENHANCEMENT OF THE LATE AFTERHYPERPOLARIZATION. S.A. Shefner and B. Tabakoff. Dept. Physiol. Biophys., Univ. of Illinois at Chicago, Health Sci. Ctr., Chicago, IL 60680.

Locus coeruleus (LC) neurons in the totally-submerged rat pontine slice preparation fire spontaneously at a rate of about 0.25-7 Hz. Bath application of ethanol (ETOH) has been shown to reversibly decrease the rate of such firing (Shefner, S.A., et al., Soc. Neurosci. Abstr.,8:651,1982). This inhibition often occurred in the absence of, or preceding ETOH-induced membrane hyperpolarization. In the present study, the size and shape of spontaneous action potentials were studied before, during and after ethanol application, to see if any changes in active membrane properties could account for the inhibition of firing.

Intracellular recordings were made from rat LC neurons, in vitro, and ETOH (1-60 mM) was applied in the bath. This concentration range corresponds to ETOH levels found in brain during mild intoxication, through levels found during ataxia and sedation. Membrane potential was monitored on a penrecorder. Action potentials were stored on paper records, pictures of oscilloscopic traces, and/or were digitized using using a Coulbourn Signamax data acquisition system and stored for later signal averaging and measurement on an IBM XT computer.

ETOH was tested 42 times on 29 LC neurons which showed stable spontaneous firing rates. All but 2 cells showed a reversible decrease in firing rate and 15 cells showed a complete block of firing in the presence of ETOH. All cells which showed a decreased firing rate also showed an increase in the duration and amplitude of the later phase of the afterpolarization (AHP), and an apparent slowing of the rate of rise of the prepotential leading up to the spontaneous action potential. In 66% of the cells, the early phase of the AHP was decreased in amplitude. Decreased spike amplitude (31% of cells) and an increase in threshold (44%) were sometimes seen in the presence of ETOH; since decreased firing could occur in the absence of these changes, they do not appear to be the main factors responsible for the ETOH-induced inhibition. The primary cause of the slowing of spontaneous firing of LC neurons, therefore, appears to be an increase in the late AHP following the spike and a decrease in rate of depolarization preceding each spontaneous spike. Both effects could result from enhancement of a long-lasting K^+ conductance.

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284.14 EFFECTS OF POSTNATAL (WEEKS 1 OR 2) ETHANOL TREATMENT IN MALE RATS. A. J. Ritchie,* M. Spencer,* B. Mesloh* and T. B. Sonderegger. Dept. of Psychol., Univ. of Nebraska, Lincoln, NE 68588-0308.

While most studies of the effects of ethanol on the developing organism have examined the effects of prenatal exposure, the study described here examines the effects of known quantities of ethanol on CNS development during the early postnatal period in the rat. Using an intragastric intubation procedure designed to counteract the effects of underfeeding (Sonderegger et al., Neurobehav. Tox. and Teratol. 4: 477, 1982), ethanol in a 30% Sustagen vehicle (Mead Johnson) was administered twice daily on postnatal days 1-7 or 8-14 in doses tapered to reach a maximum of 4 g/kg on treatment day 4.

25 litters of Charles Rivers CD albino rats, reduced to 10 pups each, were used in a split-litter design. On postpartum day 1 pups were randomly assigned to a treatment group: ethanol (E), Sustagen (S), pair-underfed (P), or handled (H). S pups received comparable volumes of isocaloric (sucrose) vehicle. Animals from additional litters were used as unhandled (U) controls. Only data from the males are reported here.

Mortality was low (10/130). Body weights were comparable on day 14 and beyond. Trials (2-min) in the open field taken on days 30-33 indicate increased activity (number of squares entered) over days ($p<0.03$) for all groups. E (treated days 1-7) animals were hypoactive relative to littermate controls ($p<0.05$), but did not differ in their latencies in initially leaving the center of the field. Other measures (percent time in center, latencies, behavioral episodes) taken in the open field differentiated between E groups as well as between E groups and controls, particularly the "Treatment x Trial" interactions. This finding differed from an earlier study in which male rats (comparably treated with ethanol on postnatal days 1-8) were found to be hypoactive when tested on day 120.

Observations from photocell activity measures on days 70-73 were also obtained and will be presented. In addition, a mating study (days 135-150) done with both groups of E animals and their H controls will be described.

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284.15 EFFECTS OF SYSTEMIC ETHANOL ON RESPONSES OF HIPPOCAMPAL UNITS TO IONOPHORETICALLY APPLIED NEUROTRANSMITTERS. J.R. Mancillas, G.R. Siggins and F.E. Bloom. Scripps Clinic and Res. Fdn., La Jolla, CA 92037

The effects of ethanol on synaptic transmission have been previously studied in our lab by evaluating changes in the responses of hippocampal pyramidal cells to stimulation of afferent pathways. We found ethanol to cause increases in both post-stimulus excitations and inhibitions. Here, we report on our efforts to determine the site of action of ethanol and the synaptic transmitters involved.

The activity of single CA1 and CA3 pyramidal cells was recorded with five-barrel microelectrodes, in rats anesthetized with halothane (0.75-1%). Ionophoretic pulses of Acetylcholine (ACH), Norepinepinephrine (NE), Serotonin (5-HT) or GABA (concentration= 0.1M, PH=4) were repeatedly applied at regular intervals. After the control responses of a unit to high and low doses of a transmitter were established, ethanol (0.75-1.5 gm/kg) was injected i.p., and the magnitude of the responses was re-evaluated periodically.

Ethanol significantly increased the responses of single neurons to ACH in 8 subjects, decreased them in 2 and had no clear, significant effect in 2 other. An increased ACH response was observed in all 5 recordings from area CA1. In CA3, an increase was seen in 3 cases, a decrease in 2 and no change in 2. Enhancement of ACH responses predominated whether they were evaluated in absolute number of spikes/sec. or as % of baseline firing rate.

No significant ethanol effects on responses to NE (n=3) or 5-HT (n=3) were observed. Ethanol seemed to cause a small but consistent potentiation of inhibitory responses to GABA in 10 subjects (CA1=8, CA3=2), a depression in 1 (CA3), and no clear change in 4 (CA1=2, CA3=2). These effects, however, were also observed in 5 of 6 saline-injected animals. Tests of 7 cells using GABA applied from 2 different barrels suggests that the apparent potentiation was an artifact of repeated iontophoretic application of GABA rather than a biological phenomenon.

Our results suggest that ethanol may potentiate post-synaptic responses to ACH in the hippocampus, and that this effect may mediate the increases in afferent excitation observed previously. Experiments to test the effects of ethanol on responses to other inhibitory transmitters are in progress. Supported by an APS fellowship to J.R.M. and the USPHS (AA 06420).

284.16 EFFECTS OF ETHANOL ON PLASMA VASOPRESSIN RELEASE IN THE RAT D. L. Colbern, J. ten Haaf*, B. Tabakoff, and Tj.B. van Wimersma Greidanus. Dept. Physiol. Biophys., Univ. Ill. Chicago Med. Ctr., P.O. 6998, Chicago, IL 60680, and Rudolf Magnus Inst. Pharmac., State Univ. Utrecht, The Netherlands

Vasopressin (VP) levels in human plasma have been shown to exhibit a complex pattern of responses to the administration of ethanol. (Helderman et al., J. Geront., 33: 39-47, 1978; Linkola et al., Acta physiol. scand., 104:180-187, 1978). In rats, ethanol has generally been thought to inhibit VP release into the peripheral circulation, however, the primary evidence for this conclusion has been indirect. We used radioimmunoassay to more accurately assess the effect of ethanol on the release of VP in this species.

Male, Wistar rats (170-215g) were maintained on a 14:10, light:dark schedule (lights on 05:00 h) and given food and water ad libitum. Rats were injected ip with 2.0 g/kg ethanol (15%, v/v) 5 or 60 min before decapitation. Control animals were given an equal volume of 0.9% saline (1.67 cc/100g) or a sham injection. Another group was left undisturbed until decapitation. Injections were started at 15:00 h and decapitations were completed by 17:15 h.

Plasma samples were extracted with Vycor glass powder and VP content was determined by radioimmunoassay using an antiserum (W1E) which had less than 0.01 % crossreactivity with oxytocin.

Results are presented in the table below. Five min after treatment, plasma VP was significantly elevated in rats given ethanol compared to those given saline ($p<.05$) or sham ($p<.01$) injections. Sixty min after injection, VP levels were lower in rats given ethanol compared to levels in sham ($p<.05$) or saline ($p<.1$) treated animals. Saline or sham injections had no effect on VP release 5 or 60 min after injection compared to VP release in untreated rats at that time of day (2.39 ± 0.45, n=13).

INJECTION		VASOPRESSIN[$] 5 min		60 min
Saline	(n=7)	3.14 ± 1.11	(7)	3.60 ± 1.09
Sham	(7)	2.49 ± 0.44	(8)	3.16 ± 0.51
Ethanol	(7)	8.96 ± 1.70**	(8)	1.58 ± 0.48*

$(pg/ml, $\bar{x}$ ± sem)

Thus, in addition to the generally held notion that ethanol administration inhibits VP release, radioimmunoassay techniques revealed that VP release in rats may also be markedly stimulated shortly after an injection of ethanol. [Supported in part by NIAAA-NRSA to DLC & NJAAA-2696 to BT]

284.17 THE EFFECT OF ETHANOL ON DOPAMINE UPTAKE IN STRIATAL SYNAPTOSOMES M.G. Hadfield Neurochemistry Research Lab., Section on Neuropathology, Dept. of Pathology, Medical College of Virginia/Virginia Commonwealth U., Richmond, Virginia 23298

Evidence is accumulating that ethanol has an important effect on chemical neurotransmission. The reports embrace a number of neurotransmitters and neurotransmitter functions. But the record is silent for dopamine (DA) uptake. In the present study, we looked at the effects of varying doses of in vitro ethanol (up to 500 uM) on tritiated DA uptake in synaptosome--rich homogenates of striatum obtained from male ICR mice. Incubation of tissue (1-3 mg original tissue/ml) was carried out for five minutes at 37°C in Krebs-Henseleit bicarbonate media under 95% O_2/5%CO_2. It was enriched with appropriate amounts of glucose, EDTA, ascorbic acid, pargyline and several concentrations of DA ranging from 0.067 to 0.2 uM. Cocaine (10 um) inhibited samples were simultaneously incubated to determine passive uptake. The net uptake values were subjected to Michaelis-Menten analysis to determine Km and Vmax. No changes in DA uptake were noted except at the highest concentration of ethanol utilized (500 uM). At that extreme dose, there was a modest but significant (25%) decrease in Vmax but still no change in Km. These results indicate that ethanol has little effect on DA uptake, at least in vitro. In view of the fact that ethanol produces fluid membrane shifts that may interfere with sodium channels and ATPase activity, we had expected a more pronounced decrease in Vmax due to metabolic uptake inhibition. Though uptake inhibition of DA in the striatum is thought simply to reflect increased release of neurotransmitter, ethanol increased DA release, in the studies of others, at concentrations only 1/5 our highest dose. These essentially negative findings indicate that we should direct our search for ethanol effects on dopaminergic mechanisms to areas other than uptake, such as DA receptor activity where a more important effect may occur.

284.18 ARE SHORT SLEEP AND LONG SLEEP MICE SPECIFICALLY SENSITIVE TO ALCOHOL? H.P. Alpern, T.D. McIntyre. Behavioral Neuroscience Program, Department of Psychology, University of Colorado, Boulder, Co. 80309

Two lines of mice selectively-bred for short and long ethanol-induced sleeptimes (SS & LS) are interesting because they seemingly support a genetic hypothesis of alcohol sensitivity. We do not challenge this notion, but, the available evidence does not support the conclusion that these lines are just sensitive to alcohols. The alcohol-specificity case hinges on a study that examined narcotic responses to ethanol, methanol, t-butanol, pentobarbital (PB), paraldehyde, chloral hydrate and trichloroethanol (Erwin et al., 1976), which found that only the alcohols separated these lines.

Other findings, however, suggest that the alcohol-induced differences are due to tonic neural activity. SS mice display lower thresholds to flurothyl-induced seizures, and in an open-field SS mice were more active. Additionally, gamma-butyrolactone, L-phenylisopropyl adenosine and chlordiazepoxide induce longer sleeptimes in LS mice. These findings contradict Erwin et al., and the finding that SS sleep longer after PB (O'Connor et al., 1982).

One alternative theory contends that continued selection pressure has altered the lines so that the SS are now PB sensitive (O'Connor et al., 1982). Due to the ambiguity regarding barbiturates we reexamined the statistical results reported by Erwin et al. Using their statistical data our reanalysis finds different results. We confirmed their results for the alcohols, but found that chloral hydrate ($t = 3.02$, $df = 37$, $p<.01$), trichloroethanol ($t = 2.16$, $df = 18$, $p<.05$) and paraldehyde ($t = 2.93$, $df = 37$, $p<.01$), induced longer sleeptimes in LS. PB induced longer sleeptimes in SS ($t = 2.99$, $df = 124$, $p<.01$). Thus, the conclusion that the two lines are ethanol-specific is not supported by the original data, while the result for PB is inconsistent with those for other CNS depressants. Since some ambiguity remains, this study was designed to ascertain sleeptimes after administration of PB, phenobarbital, and thiopental.

We report that, consistent with previous findings for ethanol, t-butanol, methanol, paraldehyde, chloral hydrate, trichloroethanol, gamma-butyrolactone, chlordiazepoxide, and adenosine, whenever the two lines were different the barbiturates pentobarbital, phenobarbital and thiopental induced longer sleeptimes in LS mice. Thus, the SS and LS mice are not specifically sensitive to the narcotic effects of alcohol but to many CNS depressants.

284.19 ETHANOL DIFFERENTIALLY MODULATES OPIATE, ALPHA ADRENERGIC AND MUSCARINIC RECEPTORS IN CULTURED NEURAL CELLS. M.E. Charness*, E.C. Cooper*, I. Diamond. Ernest Gallo Clinic & Research Center, Dept. of Neurology, University of California, San Francisco, California 94110.

Ethanol is believed to produce important effects on synaptic function by interacting with neural cell membranes. Using living, undisrupted NG108-15 cells, we have shown that acute ethanol exposure inhibits binding to the delta opiate receptor, and that after longer exposure, these cells express an increased number of opiate receptors. The presence of multiple neurotransmitter receptors in this cell line allowed us to simultaneously study the effects of short-term and long-term ethanol exposure on several receptors in the same living cells.

When added to whole cells immediately prior to the radioligands, ethanol inhibited the binding of ^{3}H-rauwolscine (RAUW), ^{3}H-diprenorphine (DPN), and ^{3}H-quinuclidinyl benzilate (QNB), antagonists respectively for the α_2-adrenergic, opiate, and muscarinic receptors. Pseudo-Hill plots for ethanol's inhibition of receptor binding showed slopes of 1.39 for RAUW), 1.34 for DPN, and 2.59 for QNB. The K_i for ethanol inhibition, calculated from the IC_{50} and the receptor K_d, was 172±20 mM for RAUW, 387±69 mM for DPN and 723± 145 mM for QNB. Thus, the 3 neurotransmitter receptors displayed differential sensitivity to the acute effects of ethanol. Scatchard analysis revealed an acute effect on K_d and no effect on B_{max}.

The effects of longer-term ethanol exposure were determined by culturing NG108-15 cells for 2 days in serum-free defined medium in the presence or absence of 200 mM ethanol. This treatment increased binding for RAUW 103%, DPN 86%, and QNB 37%. For all three receptors, increased binding reflected a predominant change in receptor B_{max}. Thus ethanol's potency for short-term inhibition of receptor binding correlated with an increase in receptor expression after longer-term exposure. Short and long-term exposure to ethanol may produce opposite biophysical changes in cell membranes. The differential receptor responses to ethanol we observe in NG108-15 may reflect differential receptor sensitivity to these biophysical events.

GABA AND BENZODIAZEPINES: BINDING II

285.1 MODULATION OF [35S]-TBPS BINDING BY BENZODIAZEPINE RECEPTOR LIGANDS: EFFECTS OF FLUNITRAZEPAM PHOTOAFFINITY LABELING. R. M. Mangano and G. T. Bautz*. Dept. of Pharmacology II, Hoffmann-La Roche Inc., Nutley, N.J. 07110.

The cage convulsant t-butylbicyclophosphorothionate (TBPS) binds specifically and with high affinity to the picrotoxin moiety of the benzodiazepine (BZ)/GABA/picrotoxin/chloride ionophore receptor complex (Squires et al. Mol. Pharm., 23, 326, 1983). Recent studies indicate that [35S]-TBPS binding is modulated by occupancy of the BZ receptor with its ligands (Supavilai and Karobath, Eur. J. Pharm., 91, 145, 1983). Since flunitrazepam photoaffinity labeling (PAL) is known to alter BZ agonist-receptor interactions, we have undertaken studies to determine the effects of BZ receptor PAL on the modulation of [35S]-TBPS binding.

Briefly, rat cortex was homogenized in 40 vols. of 50mM Na2HPO4-HCl, pH 7.4. The homogenate was centrifuged at 36,000 xg for 10 min. at 4° C. The tissue pellet was resuspended in fresh buffer and photolabeled in the presence of 30nM flunitrazepam (FLUN). Control tissue was exposed to UV light in the absence of FLUN. Treated tissue was washed 4x with 50mM Tris-HCl, pH 7.4 and frozen. [35S]-TBPS binding was performed at 25° C in 50mM Tris-citrate, pH 7.5 in the presence of various BZ receptor ligands. Binding was terminated by filtration through GF/B filters after 100 minutes.

Binding of [35S]-TBPS to control tissue was enhanced 22%, 35% and 31% by 10-8M, 10-7M and 10-6M concentrations of FLUN. However, in PAL membranes the FLUN stimulation was 0%, 8% and 22% at these same concentrations. Clonazepam (CLON) at concentrations of 10-8M, 10-7M and 10-6M stimulated [35S]-TBPS binding 28%, 36% and 37% in control tissue. In PAL membranes this stimulation was reduced to 4%, 19% and 24% at these concentrations. Thus FLUN PAL of BZ receptors appears to attenuate the BZ receptor modulation of TBPS binding and that higher concentrations of BZ agonists can partially overcome this effect. These preliminary experiments also indicate that covalent incorporation of FLUN into the BZ receptor by PAL per se causes a 20% stimulation of [35S]-TBPS binding.

285.2 THE BENZODIAZEPINE RECEPTOR INTERACTIONS OF A VERY HIGH AFFINITY PYRROLOBENZAZEPINE, 3-H Ro 22-8515. G. Bautz*, N. M. Spirt*, R. A. O'Brien. Dept. of Pharmacology II, Hoffmann-La Roche Inc., Nutley, N. J. 07110.

In vitro receptor binding analyses of a series of benzazepine analogs led to the discovery of Ro 22-8515, 8-Chloro-6-(2-chlorophenyl)-1,4-dihydropyrrolo=[3,4-d] [2] benzazepin-3(2H)-one (Dr. E. Trybulski), which exhibited very high affinity for the benzodiazepine (BZ) receptor. The compound has an IC 50 value of 0.0045 nM against 3H-diazepam (3H-DIAZ) and displayed a very shallow displacement curve using rat cerebral cortical membranes as a receptor source. The Hill coefficient is 0.4 in assays run at 4° C. The binding was selective for central BZ receptors since it is inactive in displacing 3H-DIAZ from peripheral kidney binding sites. When tested in the 3H-Ro 15-1788 binding assay, the affinity of the drug was greatly enhanced by GABA (IC 50 ratio of 4.1) suggesting very strong agonist activity. Ro 22-8515 is also very potent when evaluated in ex vivo 3H-DIAZ experiments; ED 50=0.26 mg/kg (DIAZ ED50=24 mg/kg.) The binding of 3H-Ro 22-8515 to rat brain synaptic membranes verified the initial observations made in the 3H-DIAZ binding assays. Binding is specific, saturable and at 100pM required incubation times of 10 or 90 minutes at 37 or 4° C, respectively, to reach equilibrium. Scatchard analyses revealed Bmax values of 60 pmols/g. wet wt. in rat cortex which are comparable to the Bmax values reported for BZ binding. At 37° C 3H-Ro 22-8515 is displaceable by DIAZ (IC50=3.8nM) and clonazepam (IC50=0.27 nM). The peripheral BZ, Ro 5-4864, and the nonBZ Zopiclone produce insignificant inhibition of 3H-Ro 22-8515 binding. The total specific binding of 3H-Ro 22-8515 is greater at 4 than 37° C, however when dissocation experiments were carried out, only 50% of the bound 3H-Ro 22-8515 is reversibly bound at 4° C, while nearly 100% is dissociable at 37° C. 3H-Ro 22-8515 might be a useful ligand for in vivo characterization and localization of benzodiazepine receptors. A preliminary evaluation using in vivo autoradiography has been completed.

285.3 CHARACTERIZATION OF BENZODIAZEPINE BINDING SITES AFTER SHORT WAVE PHOTOAFFINITY LABELING. W.F. Herblin and C.C. Mechem*. E.I. du Pont de Nemours & Co., Experimental Station, Central Res. & Devel. Dept., Wilmington, DE 19898.

We have previously shown that short-wave ultraviolet irradiation (254 nm) is more efficient than long-wave for the photo-affinity labeling of benzodiazepine sites with flunitrazepam (FZ). Under the conditions used, over 50% of the available "central type" sites from rat cortex or cerebellum could be labeled with ^{3}H-FZ. To better understand the relationship of agonist and antagonist benzodiazepine sites, we have photolabeled membranes from rat cortex in the presence of non-radioactive FZ and examined the binding of ^{3}H-ethyl-beta-carboline (BCCE) and its inhibition by BCCE, FZ, and Cl 218,872.

Rat cortical tissue was homogenized in 50 mM TRIS, pH 7.4, and centrifuged at 40,000 x g for 30 minutes. The pellet was resuspended in TRIS and washed twice by centrifugation and resuspension. The homogenate was incubated for 30 minutes at 0-4°C with 10 nM FZ and then irradiated at 254nm for 15 minutes. The tissue was washed three times by centrifugation and resuspension and used in standard binding assays. Control tissue was either incubated with FZ and not irradiated or irradiated in the absence of FZ.

In photolabeled membranes the binding of BCCE and its self inhibition are unchanged. The values for Bmax, Kd, and Ki were identical to those determined with control membranes. The inhibition of ^{3}H-BCCE by FZ shows two distinct components. Roughly half of the BCCE binding is inhibited by FZ with moderate potency (Ki~50 nM), while the remainder is virtually unaffected (Ki > 1000 nM). Cl 218,872 appears to interact with all of the BCCE sites uniformly, but the affinity is reduced 8-10 fold compared to the affinity determined in control membranes.

285.4 SOLUBLE GABA-BENZODIAZEPINE RECEPTOR COMPLEX CONTAINS SITES FOR ANIONS AND DIVALENT CATIONS, M. Gavish, M. Awad* and F. Fares*, Rappaport Inst., Dept. Pharm., Fac. Med., Technion, Haifa 31096, Israel.

Benzodiazepine receptors were solubilized from calf brain cortex by 2% deoxycholate plus 1 M NaCl and dialyzed for salt removal. As is the case with membrane-bound benzodiazepine receptors, the binding of ^{3}H-flunitrazepam is increased by GABA, NaCl and $CaCl_2$. Salt and GABA removal by dialysis decreased soluble benzodiazepine receptor binding and increased receptor sensitivity to heat treatment.

GABA (15 μM) and NaCl (50 mM) protect 30% and 20% of soluble benzodiazepine receptors respectively from heat inactivation. Combinations thereof protect 60% of binding activity from the same treatment. 5 mM of $CaCl_2$ was also found to protect 20% of benzodiazepine binding activity from heat and in combination with 15 μM GABA - the protection is 50%.

These results indicate that the sites for anions and divalent cations which were discovered in the membrane-bound state of the GABA-benzodiazepine receptor complex are retained after solubilization by deoxycholate plus NaCl.

285.5 SPECIFIC EFFECTS OF SHORTWAVE ULTRAVIOLET IRRADIATION ON THE ANION RECOGNITION SITE OF BENZODIZAEPINE/GABA RECEPTORS. R.R. Trifiletti, A.M. Snowman and S.H. Snyder. Johns Hopkins University, Dept. of Neuroscience, Sch. of Med., Baltimore, MD 21205.

(^{3}H)Muscimol has been successfully photoincorporated into rat brain GABA-A receptors by shortwave (254 nm peak output) irradiation. Salient features of photoaffinity labelling of GABA-A receptors with (^{3}H)muscimol will be presented. A natural question which arises is whether the shortwave irradiation required to obtain (^{3}H)muscimol incorporation has any effects per sè on the benzodiazepine/GABA-A complex.

Shortwave ultraviolet irradiation of rat brain water-lysed crude P2 membranes maintained at 0°C produces a dramatic, time-dependent loss of specific (35S)tert-butylbicyclophosphorothionate ((35S)TBPS) binding to sedative/convulsant receptors. Under the same conditions, neither benzodiazepine receptor binding nor GABA-A receptor binding is affected. The action spectrum for the inactivation of (35S)TBPS binding shows a maximum at about 280 nm, consistent with the involvement of tryptophan or tyrosine residues. As (35S)TBPS binding is almost entirely dependent on Eccles anions, abolishment of (35S)TBPS binding might be due to direct effects on either the TBPS or anion recognition sites. Further study revealed that shortwave irradiation also abolishes anion, barbiturate and pyrazolpyridazine (etazolate) enhancement of (^{3}H)diazepam binding, each with a similar time course as inactivation of (35S)TBPS binding. All of these effects are dependent upon Eccles anions, and we therefore propose that the primary influence of shortwave ultraviolet irradiation on the benzodiazepine/GABA-A receptor is on the anion recognition site. GABAergic regulation of benzodiazepine agonist binding, which is not absolutely dependent upon anions, is largely unaffected by shortwave irradiation.

Gel filtration of soluble (^{3}H)Ro-15-1788 binding to detergent solubilized extracts of control and shortwave irradiated membranes suggests that irradiation produces a decrease in apparent size of the soluble benzodiazepine/GABA-A receptor complex. This preliminary result suggests that the anionophore function of the receptor complex may be on a distinct polypeptide linked to benzodiazepine and GABA binding subunit(s) by a single uv-labile (disulfide?) bond.

285.6 CHARACTERIZATION OF BENZODIAZEPINE BINDING SITES IN THE RAT PINEAL GLAND. E. Matthew, M. Blain* and K. Machika*. Montreal Neurological Institute, McGill University, Montreal PQ. H3A 2B4.

These studies were based on an earlier report in which we described augmentation of norepinephrine-stimulated N-acetyltransferase activity by benzodiazepines in the rat pineal gland (J. Pharmacol. Exptl. Ther. 228:434-438). Studies of the binding of five benzodiazepine ligands, (^{3}H)diazepam, (^{3}H)flunitrazepam, (^{3}H)Ro-5-4864, (^{3}H)Ro-15-1788 and (^{3}H)methylclonazepam, were carried out in homogenate preparations of pineal glands taken from male Sprague-Dawley rats (100-150gm).

The binding of (^{3}H)diazepam, (^{3}H)flunitrazepam and (^{3}H)Ro-5-4864, was reversible saturable and proportional to the amount of protein used for the assay. With (^{3}H)Ro-15-1788, a putative antagonist of the clonazepam-sensitive "central" receptor, some reversible, but non-saturable binding occurred at radioligand concentrations greater than 10nM. There was no binding of (^{3}H)methylclonazepam.

The binding of (^{3}H)Ro-5-4864 was of higher affinity (Kd=6.4nM) than that of (^{3}H)diazepam (Kd=42.8nM) or (^{3}H)flunitrazepam (Kd=68.7nM). Maximum binding sites (B_{max}) for (^{3}H)Ro-5-4864 (9.8 pmol/mg protein), (^{3}H)diazepam (6.8 pmol/mg protein) and (^{3}H)flunitrazepam (13.9 pmol/mg protein) were 30-fold, 6-fold and 9-fold higher respectively than that reported in rat brain. (Marangos et al, 1982, Braestrup and Squires, 1977 and Mohler et al, 1980). To further characterize these binding sites, IC_{50} values (concentrations required to produce 50% inhibition of radioligand binding) were determined:

	(^{3}H)Ro-5-4864	(^{3}H)Flunitrazepam	(^{3}H)diazepam
	IC_{50}(nM)		
Diazepam	12	45	-
Flunitrazepam	30	-	22
Ro-5-4864	-	4	42
Ro-15-1788	>3000	700	>3000
Clonazepam	>3000	>3000	>3000

These data demonstrate that the benzodiazepine binding sites in the rat pineal gland are of the Ro-5-4864-sensitive "peripheral"type.

285.7 REDUCED POTENTIATION BY GABA OF BENZODIAZEPINE RECEPTOR BINDING IN BRAIN CELL CULTURE AFTER CHRONIC BENZODIAZEPINE EXPOSURE. G.D. Schiller* and D.H. Farb. Dept. of Anatomy & Cell Biology, SUNY Downstate Med. Ctr., Brooklyn, NY 11203

Benzodiazepines (BZD) bind with high affinity to membrane homogenates of embryonic chick brain and spinal cord, potentiating GABA induced increases in membrane conductance (Life Sci.33(1983)2061). Down regulation of BZD receptor (BZD-R) number was observed in rat brain homogenates after i.p. administration of flurazepam (FZ) for 7d (Life Sci.23 (1978)1153). Here, we show that chronic FZ treatment has virtually no effect on basal [3H]flunitrazepam ([3H]FNZM) binding, but rapidly (within 24h) reduces GABA-enhancement of BZD binding to living primary embryonic chick brain cell cultures and cell homogenates. Cultures were exposed to FZ (100uM,48h), collected, homogenized and washed 8X by centrifugation. GABA (10uM; EC50 1uM) enhanced [3H]FNZM binding (0.5nM, 0C, measured by filtration assay) 90% in control cells, whereas enhancement was only 20% in treated cultures. Potentiation by 1mM GABA was inhibited by 100uM bicuculline. Neither [3H]FNZM binding (control:treated, 180+/-28:160+/-36, fmol/mg protein), nor affinity (ca. 7nM) were significantly altered by FZ exposure. Reduced potentiation was also observed for binding to intact cells (16mm dishes): Brain cells were exposed to FZ (100uM,24h,37C) and washed (2h,37C then 2 X 45min,23C) prior to assay. [3H]FNZM binding (1nM,30min,0C) was determined in the presence of increasing [GABA], and non-specific binding (+ 2uM FNZM) was subtracted. Reactions were terminated by aspiration of the incubation mixture and the cells washed (10sec, 2ml,0C). Membrane dilution experiments showed no effect of residual FZ on the binding assay. GABA (10uM) potentiated [3H]FNZM binding to living neurons 55+/-4% (3 expts.), was bicuculline sensitive and had an EC50 for GABA of ca. 1uM. Exposure to 100uM FZ decreased GABA potentiation to 12+/-3% above control, approx. a 5-fold decrease. A smaller effect was observed with 0.1uM FZ, and little effect was observed after a 4h exposure to 100uM FZ, indicating that reduced potentiation is both time- and dose-dependent. Interestingly, reduced potentiation was recently reported for adult rat brain but required 3wk of diazepam treatment (Nature 308(1984)74). Reciprocal enhancement by GABA and BZD's of their binding is well documented and the rapid reduction in the abilty of GABA to modulate [3H]FNZM binding may reflect an underlying mechanism involved in GABA/BZD-R sensitivity, such as may be involved in tolerance to chronic BZD treatment. (Supported by NIH NS-18536 & New York Heart).

285.8 ^{3}[H]Ro 15-1788 (BENZODIAZEPINE ANTAGONIST) BINDING TO MOUSE BRAIN IN VIVO: MARKED ENHANCEMENT BY GABA AGONISTS, ADENOSINE AGONISTS, CALCIUM CHANNEL BLOCKERS AND CANNABIMIMETICS. B. K. Koe and E. Kondratas*. Central Research, Pfizer Inc., Groton, CT 06340

Benzodiazepine receptors, GABA receptors and picrotoxin binding sites constitute a complex which regulates the chloride ion channel. GABA agonists and agents which facilitate ^{3}[H]GABA binding enhance the binding of ^{3}[H]benzodiazepines to brain membranes in vitro and in vivo. Although GABA agonists increase ^{3}[H]diazepam or ^{3}[H]flunitrazepam (^{3}[H]FNP) binding, they have no effect on that of the benzodiazepine antagonist, ^{3}[H]Ro 15-1788, in vitro. This differential effect is the basis of a simple test to distinguish between benzodiazepine agonists and antagonists.

Unexpectedly, we found that mice pretreated with progabide (320 µmol/kg) or Na valproate (1 mmol/kg) showed marked enhancement of ^{3}[H]Ro 15-1788 (100 µCi/kg i.v.) binding in vivo (288% and 556% of control, respectively). Cartazolate (100 µmol/kg) and the cannabimimetic, levonantradol (1 µmol/kg), also caused an increase in in vivo ^{3}[H]Ro 15-1788 binding (198% and 269% of control, respectively). In addition, we found that the adenosine agonist, N^{6}-cyclohexyladenosine (10 µmol/kg), and the Ca^{+2} channel blocker, nimodipine (100 µmol/kg), elevated binding of i.v. ^{3}[H]Ro 15-1788 to mouse brain (366% and 225% of control, respectively). These increases in ^{3}[H]Ro 15-1788 binding in vivo were higher than the corresponding increases in ^{3}[H]FNP binding. In ^{3}[H]Ro 15-1788 binding, the relative increases in pellet (P) and homogenate (H) radioactivity were about the same. As a result, "fraction bound" (P/H) did not vary by much, in contrast to increases or decreases in fraction bound observed in ^{3}[H]FNP binding with binding enhancers or inhibitors, respectively. The enhancement of ^{3}[H]Ro 15-1788 binding in intact mice by progabide, N^{6}-cyclohexyladenosine and nimodipine is reminiscent of their effects on regional cerebral blood flow. These drugs probably increase the latter via vasodilatation upon stimulation of the respective receptors on cerebral blood vessels (Edvinsson et al., 1980). Thus, enhanced ^{3}[H]Ro 15-1788 binding may result from the facilitated entry into brain of radioligand which then binds avidly to benzodiazepine receptors. In vivo ^{3}[H]Ro 15-1788 binding may be a useful method of ascertaining the effect of drugs on cerebral blood flow in regions containing benzodiazepine receptors.

285.9 CONVULSANT/BARBITURATE RECEPTORS ARE PART OF THE GABA-BENZODIAZEPINE RECEPTOR PROTEIN COMPLEX. R.G. King, G.B. Stauber, J.B. Fischer, and R.W. Olsen, University of California, Riverside, and UCLA School of Medicine, Los Angeles, CA 90024.

Binding activity for the cage convulsant [^{35}S]t-butylbicyclophosphorothionate (TBPS) was solubilized from rat brain membrane with the detergent 3-[(3-cholamidopropyl) dimethylammonio] propanesulfonate (CHAPS) and shown to copurity with the GABA/benzodiazepine complex. [^{35}S]TBPS binding activity in membranes and CHAPS extracts was inhibited by cage convulsants, picrotoxin-like convulsants, barbiturates and related depressants. The binding activity was also allosterically inhibited by GABA receptor ligands in a bicuculline-sensitive manner. [^{35}S]TBPS binding activity was solubilized in 50% yield using 1% CHAPS, giving the following binding properties: Kd=26 nM, Bmax=0.4 pmol/mg protein. Gel filtration chromatography on Sepharose 6B in 0.5% CHAPS gave a single peak of [^{35}S]TBPS binding which co-migrated with [^{3}H]muscimol binding and [^{3}H]flunitrazepam binding with an apparent molecular weight of about 900,000. The BZ binding activity showed a 20% enhancement with 1 mM Pentobarbital. [^{35}S]TBPS binding activity can be retained and copurified with barbiturate-enhanced [^{3}H]flunitrazepam and [^{3}H]muscimol binding activity on a benzodiazepine affinity column. This work provides further evidence for the existence of the GABA-BZ-convulsant/barbiturate receptor together as a single protein complex.

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285.10 PURIFICATION OF THE GABA/BENZODIAZEPINE/BARBITURATE RECEPTOR COMPLEX FROM RAT BRAIN. J.B. Fischer, G B. Stauber*, R.G. King, & R.W. Olsen. University of California, Riverside, and UCLA school of Medicine and Brain Research Institute, Los Angeles, CA 90024.

Receptor binding activity for the inhibitory neurotransmitter γ-aminobutyric acid (GABA) was purified several hundred-fold to near homogeneity with retention of modulatory receptor sites for benzodiazepines (BZ) and barbiturates. GABA receptor ([^{3}H]muscimol), BZ receptor ([^{3}H]flunitrazepam), and convulsant-barbiturate receptor ([35S]t-butyl bicyclophosphorothionate, TBPS) binding activities, solubilized from rat brain with the detergent deoxycholate, were all retained (>90%) on an affinity column of agarose-immobilized BZ ligand (RO7-1986) in Triton X-100, while essentially all the protein passed through. Free flurazepam eluted all three activities with virtually no protein. Purified [^{3}H]muscimol binding activity (yield 60%, specific activity 600-1800 pmol/mg protein) was inhibited by GABA receptor-specific analogs and enhanced 50% by 1 mM pentobarbital (after removal of flurazepam by dialysis and/or DEAE column). This also allowed detection of purified [35S]TBPS binding, inhibited by picrotoxin, cage convulsants, and barbiturates. Brain-specific [^{3}H]flunitrazepam binding was also purified and this ligand could be incorporated covalently by UV light exposure. The photoaffinity ^{3}H-labelled protein gave a single peak on gel filtration chromatography (mol. wt. about 200,000) which co-migrated with another purified sample labelled with 125I. SDS gel electrophoresis revealed four bands at 46-66,000 by silver stain, Coomassie stain, and 125I (nonspecific labelling), one of which was also labelled with ^{3}H (flunitrazepam photoaffinity labelling). This purification to near homogeneity indicates that the mammalian brain GABA receptor-chloride ion channel exists as a protein complex bearing modulatory sites for the benzodiazepines and barbiturates.

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285.11 PURIFICATION AND PROPERTIES OF THE GABA/BDZ COMPLEX. John F. Tallman, Department of Psychiatry, Yale University School of Medicine, New Haven, CT. 06508

An affinity column suitable for the purification of the benzodiazepine receptor has been prepared by linking 3-aminoclonazepam to agarose using a 15 carbon, hydrophilic spacer. High-affinity binding sites for GABA and the benzodiazepines were solubilized using the detergent Lubrol-PX and adsorbed to the affinity column. The column was washed with the detergent CHAPS and eluted with flurazepam in CHAPS. The eluted material contains two high-affinity binding sites for GABA (6nM and 300nM) and one lower affinity site which can be monitored by the GABA-ergic enhancement (>1uM) of benzodiazepine binding to the purified and dialyzed preparations. In addition to the benzodiazepines, high-affinity binding of β-carboline ethylester and Ro15-1788 can be demonstrated and the number of these sites correlates with the number of high-affinity GABA receptors. All of the benzodiazepine ligands enhance the binding to the high-affinity GABA site by increasing the apparent number of these sites, at the expense of the lower affinity GABA site.

In contrast to the enhancement of GABA binding by the benzodiazepine ligands, reducing agents (dithiothreitol) decreased binding to the high affinity GABA receptor indicating the possible involvement of sulfhydryl bonds in the maintenance of receptor binding site integrity. Thiocyanate, previously shown to inhibit binding to high-affinity GABA sites in membranes, eliminates the high affinity component of GABA binding in the purified preparations.

Analysis of the protein content of the purified fractions by SDS gel electrophoresis under reducing conditions indicates the presence of several major bands with molecular weight close to 50,000 daltons. Additional bands of molecular weight near 95,000 daltons are also noted. The hypothesis that these bands are normally a part of the GABA/BDZ complex is under investigation. (Supported by NIMH Grant MH38813 and the State of Connecticut).

285.12 MULTIPLE BENZODIAZEPINE RECEPTORS ARE PRESENT IN THE HUMAN BRAIN. G. Biggio, M. Serra*, A. Concas*, S. Mele*, S. Montaldo*, and M.G. Corda. Institute of Biology, Chair of Pharmacology and Institute of Forensic Medicine, University of Cagliari, Italy.

Several lines of evidence have suggested that at least two distinct subclasses (Type I - Type II) of benzodiazepine recognition sites are present in the mammalian brain. Type I recognition sites were suggested as those most sensitive to the β-carboline and triazolopyridazine derivatives while Type II recognition sites were those with lower sensitivity to the above drugs. On the basis of these findings the aim of our study has been to investigate whether the two subclasses of benzodiazepine recognition sites are present in human brain. For this purpose we studied the kinetic characteristics of ^{3}H-FNT and ^{3}H-β-CCe binding in membranes from three different areas (cerebral cortex, cerebellum and hippocampus) of the human brain. Moreover, to selectively identify the Type II sites, we evaluated ^{3}H-FNT binding in the presence and absence of CL-218872, a drug which binds almost exclusively to Type I benzodiazepine recognition site. As revealed by the Scatchard plot analysis the total number of binding sites labelled by ^{3}H-β-CCE was markedly lower than that labelled by ^{3}H-FNT. In fact, only 50% of the binding sites for ^{3}H-FNT were also available for ^{3}H-β-CCE. This finding indicates that in the cerebral cortex, hippocampus and cerebellum of the human brain at least 50% of benzodiazepine recognition sites is that of Type II. This conclusion is further supported by the evidence that CL-218872 (5×10^{-6}M) inhibited by 50% ^{3}H-FNT binding in membranes from the above brain areas. The results suggest that two distinct types of benzodiazepines recognition sites are present in different areas of the human brain.

GABA AND BENZODIAZEPINES: BIOCHEMISTRY

286.1 BENZODIAZEPINE INFLUENCE ON NOREPINEPHRINE RELEASE: REGIONAL SPECIFICITY. C.K. Kellogg, T.M. Retell and N. Harary. Department of Psychology, University of Rochester, Rochester, NY 14627.

The results of the present studies indicate that DZ can profoundly affect NE release in specific brain regions, and this effect is mediated via the BZ-GABA receptor complex. In the first study, DZ was administered to Long Evans rats over days 13-20 of gestation in doses of 1.0, 2.5, or 10.0 mg/kg/day. The offspring were studied beginning at 70 days of age. The hypothalamus, hippocampus and cerebellum were dissected out for analysis of NE release in vitro. Tissues were initially incubated with ^{3}H-NE (10^{-7}M) at 37°C for 20 min. Following a series of rinses, release was induced by incubation of the tissue in medium containing 25mM KCl. The evoked release (difference between the percent tissue ^{3}H-NE released in high potassium or physiologic medium) in the hypothalamus was 11.5±1.5% in uninjected control rats and reduced 28%, 32%, and 64% in animals exposed prenatally to DZ at 1.0, 2.5, or 10.0 mg/kg respectively. Prenatal DZ had no effect on NE release from the hippocampus or cerebellum. In the hypothalamus, incubation of the tissue with the alpha receptor antagonist phentolamine (10^{-5}M) induced increases in the evoked release in all groups. The magnitude of the increase, however, was greater in the drug exposed groups than the uninjected rats; 353% in animals exposed to DZ at 10 mg/kg prenatally and 124% in the controls. In the second study, the hypothalamus and cerebellum of uninjected adult rats were incubated in the presence of DZ (10^{-5}M) or DZ plus biccuculine (10^{-5}M), a GABA receptor antagonist. DZ added in vitro decreased the evoked release of 3H-NE from the hypothalamus but not from the cerebellum. The reduction in release induced by DZ in the hypothalamus was prevented by biccuculine. In the cerebellum, biccuculine increased the release slightly over basal release. It thus appears that in the hypothalamus, the BZ binding site is linked to the GABA receptor and may be present on presynaptic NE terminals. In the cerebellum, while BZ binding sites may be present on NE neurons (Neurosci. Lett. 27:199, 1981), they are not capable of exerting the same control over NE transmitter function as in the hypothalamus. Work supported by Grant no. MH31850.

286.2 ARE PERIPHERAL TYPE BENZODIAZEPINE RECEPTORS COUPLED WITH CALCIUM CHANNELS? G. Le Fur, M. Mestre*, T. Carriot* and A. Uzan. PHARMUKA Laboratoires, Groupe RHONE POULENC SANTE, 35, quai du Moulin de Cage, 92231 Gennevilliers, France.

In the guinea pig papillary muscle it has been possible to characterize electrophysiological and pharmacological responses coupled with benzodiazepine (BZ) binding sites of the peripheral type. This response is characterized by a decrease in the duration of the action potential whereas the amplitude remains unchanged, and a decrease in contractility. It fulfils with all the following criteria: the order of potency of the agonists is RO5-4864 > diazepam > clonazepam, the effect of RO5-4864 is GABA-independent, antagonized by the selective antagonist of the peripheral type BZ binding sites PK 11195 but not by the antagonist of the brain type BZ binding sites RO15-1788. PK 11195 also antagonized the increase in the duration of action potential provoked by the calcium channel agonist BAY K-8644 but not that induced by the potassium channel blocker tetraethylammonium. The decrease in the duration of action potential obtained by addition of different calcium channels blockers like nitrendipine, verapamil and diltiazem was antagonized by PK 11195. In conclusion PK 11195 which has no effect by itself on the duration of the action potential but antagonized the effects of agonist or antagonists of the calcium channels might favor the resting state of these channels. Moreover as the effects of RO5-4864 were reversed by addition of $CaCl_2$, we suggest that peripheral type BZ receptors are coupled with calcium channel at least in the guinea pig papillary muscle.

286.3 γ-AMINOBUTYRIC ACID MODULATES THE FUNCTION OF ACETYLCHOLINE RECEPTORS IN ADRENAL MEDULLA. Y. Kataoka*, I. Hanbauer[1], Y. Gutman*, A. Guidotti and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032 and [1]Hypertension Endocrine Branch, NHLBI, NIH, Bethesda, M.D. 20205.

The chromaffin cells of adrenal medulla contain γ-aminobutyric acid (GABA), glutamic acid decarboxylase, GABA aminotransferase and GABA receptors. These receptors modulate the acetylcholine-induced release of catecholamine (CA) in primary cultures of chromaffin cells. In these cultures 1 μM of bicuculline (GABA antagonist) facilitates the release of CA caused by nicotinic receptor stimulation but fails to change the release of CA evoked by KCl depolarization (Proc. Natl. Acad. Sci. USA 81, 1984, in press).

Since opioid peptides coexist with CA in the vesicles of chromaffin cells and nicotinic receptor stimulation releases both neuromodulators, we have investigated whether bicuculline changes the release of met^5-enkephalin like peptide (met-enk) elicited by nicotine. Bicuculline (1 μM) inhibited the release of met^5-enkephalin by various doses of nicotine changing the spontaneous release of met-enk.

To evaluate the physiological role of GABA in medullary function in vivo, the release of CA from adrenal medulla was studied in anesthetized (pentobarbital 0.43 μg/kg i.v.) American Foxhound dogs weighing 20-25 kg. After the transection of splanchnic nerve, blood was collected from the lumbar adrenal vein and drugs were injected through the cannula placed on the femoral vein. Plasma CA were isolated by Al_2O_3 adsorption and measured by HPLC coupled to an electrochemical detector. THIP (GABA agonist) (5 mg/kg) decreased the release of CA evoked by splanchnic stimulation (10 V/3-6 Hz), while bicuculline (0.5-1.0 mg/kg) increased it. The effects caused by THIP and bicuculline were more pronounced on the release of epinephrine than in that of norepinephrine.

These results support the view that intrinsic GABAergic mechanisms may modulate chromaffin cells responsiveness to incoming cholinergic stimuli.

286.4 THE MECHANISMS OF GABA TRANSPORT ACROSS THE SYNAPTOSOMAL PLASMA MEMBRANE. M.B. Troeger*, D.F. Wilson* and M. Erecinska*. (SPON: M. Reivich) Dept. of Pharmacology, Univ. of Penn., Philadelphia, Pa. 19104.

The role of homoexchange in gamma-aminobutyric acid (GABA) transport was studied in synaptosomes isolated from rat mid-brain and cortex. The stimulation of ^{14}C GABA efflux by external GABA (GABA homoexchange) followed Michaelis-Menten kinetics with respect to the external GABA concentration ($[GABA]_e$). The $[GABA]_e$ required for half-maximal stimulation of ^{14}C GABA efflux (K_{app} for homoexchange) was 7.25 ± 0.91 uM and the values for V_{max} at 30, 60, 90 and 120 sec were 2.1 ± 0.3, 1.5 ± 0.1, 1.3 ± 0.1 and 1.2 ± 0.1 nmoles/mg protein/min, respectively. The dependence of homoexchange on external sodium ion concentration ($[Na^+]_e$) was determined by measuring the number of counts liberated from synaptosomes preloaded with ^{14}C GABA and diluted into media containing 100 uM cold GABA and various $[Na^+]_e$. Homoexchange was inhibited by 35% at 15 mM Na^+, compared to the control at 140 mM Na^+. GABA fluxes in both directions (ie. uptake, release, and homoexchange) were determined by preloading the synaptosomes with ^{14}C GABA and diluting them into media containing ^{3}H GABA and $[K^+]_e$ from 2.5 to 60 mM. The total concentrations of GABA in the pellets and supernatants were measured by HPLC in order to evaluate the specific activity of the labeled amino acid. Net uptake of GABA at 2.5 and 5 mM K^+_e was 10 to 25% of the total influx at 3 min and 40 to 55% at 15 min. Depolarization by increasing $[K^+]_e$ to 20 to 60 mM decreased uptake (V_{max} was 60% of control), increased release and had a negligible effect on homoexchange. Our results indicate that GABA homoexchange is an intrinsic property of the transport of this amino acid by a carrier protein across the synaptosomal plasma membrane. The contribution of homoexchange to the total flux depends on the concentration of sodium, potassium and GABA in the external and internal environments.
Supported by grant NS 14505 from NIH.

286.5 INACTIVATION OF GLUTAMIC ACID DECARBOXYLASE BY γ-ACETYLENIC GABA IN RAT SUBSTANTIA NIGRA, STRIATUM, AND RETINA. J.F. Cubells, J. Blanchard*, D.M. Smith*, and M.H. Makman* Depts. of Neuroscience, Biochemistry and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461

4-Amino, 5-hexynoic acid (γ-acetylenic GABA; γ-AG) is a mechanism-based irreversible inhibitor of several pyridoxal phosphate-linked enzymes, including GABA-transaminase, ornithine-δ-aminotransferase and glutamic acid decarboxylase (GAD). Rando (J. Biol. Chem. 256:1111, 1981) has shown, however, that ^{3}H-γ-AG specifically labels mouse brain GAD in vivo when it is co-administered with gabaculine. We report here studies of the in vivo inactivation of GAD by γ-AG in rat substantia nigra (SN), striatum and retina.

Rats were stereotaxically injected unilaterally with 6.5 μg γ-AG in .25 μl and killed at intervals thereafter from 15 minutes to 14 days. SNs were rapidly dissected and frozen. GAD activities were assayed in Triton-broken homogenates by measuring the $^{14}CO_2$ released from 1-^{14}C-glutamate. The ratio of GAD activities in injected vs. noninjected SNs (R/L) was used as an index of GAD activity remaining in the injected SN. R/L dropped to .43 by 1 hour, .16 by 4 hours and was .24 at 21 hours. R/L was .60 at 7 days, and .89 at 14 days. Vehicle injections did not affect GAD activities. R/L was .39, .59 and .87 1 hour after injection in 3 rats which received 6.5 μg, .65 μg, or .065 μg intranigral γ-AG, respectively.

Intrastriatal γ-AG produced inactivation of striatal GAD which depended on the distance of the striatal tissue from the injection site. Two rats received 6.5 μg γ-AG in the mid-striatum and were killed 1 hour later. Striata were removed and sliced into anterior, middle and posterior portions, such that the injection was in the middle portion. R/Ls were .95, .55 and .84, respectively.

Retinal, nigral and striatal GAD activities were assayed from rats which received H_2O, 50, or 100 mg/kg γ-AG s.c., and were killed after 4 hours. Retinal GAD was inactivated to a greater extent (18% and 12% of control activity at 50 and 100 mg/kg respectively) than either nigral (95% and 82%) or striatal (92% and 72%) GAD.

In summary, intranigral γ-AG produces time- and dose-dependent loss of SN GAD activity, which slowly recovers to control levels. Intrastriatal γ-AG affects striatal GAD similarly. Finally, retinal GAD is more effectively inactivated than SN or striatal GAD by peripheral γ-AG.

γ-AG was a gift from Merrell. (Supported by USPHS Grants NS-09649, EY-04633 and 5T32 GM-07260.)

286.6 GABA AND PIPECOLIC ACID: POSSIBLE RECIPROCAL MODULATION. M.d.C. Gutierrez, E. Giacobini, Dept. Pharmacology, Southern Illinois Univ. School of Medicine, Springfield, IL 62708.

Pipecolic acid (PA, piperidine-2-carboxylic acid) is the major product of lysine metabolism in the mammalian brain (Giacobini, E. et al., Cell Mol. Biol., 26:135, 1980). We have characterized the binding of ^{3}H-PA and its distribution to P_2 fraction membranes of mouse brain (Giacobini, E. and Gutierrez, M.d.C., Glutamine, Glutamate and GABA in CNS, Alan Liss Publ., pp. 571-580, 1983). The binding was found to be saturable (70 nM), temperature and Na^+ dependent. A high affinity binding site with an apparent K_D of 33.2 nM and a B_{max} of .2 pmol/mg protein was demonstrated. The regional distribution of ^{3}H-PA specific binding in mouse brain showed the highest concentration in cerebral cortex, thalamus and olfactory bulb. Unlabeled PA (10^{-3}-10^{-11}M) displaced specific binding of ^{3}H-PA in a concentration dependent manner. Out of several substances tested, only proline showed a similar pattern of displacement. Pre-incubation of the membrane preparation with GABA (10^{-3}-10^{-11}M) resulted in either an increase or decrease of ^{3}H-PA binding depending on the concentrations of GABA and PA. These results suggest a modulatory action of GABA on PA binding sites. On the other hand, PA has been demonstrated by us to increase GABA release from brain slices and to decrease GABA uptake in synaptosomes and glia. Physiologically this may lead to an amplification of GABA action. In order to further characterize the GABA-PA relationship we tested several compounds affecting GABA receptor function on PA binding. The postnatal development of ^{3}H-PA specific binding was studied in the whole brain of the mouse. ^{3}H-PA binding increased progressively (8-fold) from day one after birth to 16 days. Following this developmental peak, the binding decreased gradually to 30 days at which age, adult values were attained. The ontogenetic relation between the concentration of ^{3}H-PA binding sites and PA endogenous concentration in brain will be discussed. (Supported by SIU Central Research Grant 2-40202 to E.G.)

286.7 PALLIDAL AND NIGRAL IRON CONCENTRATION REDUCED BY GAMMA-VINYL GABA. J.M. Hill* (SPON: P.D. MacLean). Lab. of Brain Evolution and Behavior, NIMH, Bethesda, MD. 20837.

Recent histochemical studies have indicated that there is considerable overlap of brain areas accumulating iron in oligodendrocytes and in neuropil with those in which GABA systems terminate (Hill and Switzer, Neurosci., 11, 595-603, 1984). The ventral pallidum, globus pallidus, substantia nigra, pars reticulata, and cerebellar nuclei are iron rich areas receiving GABA containing efferents, and are among the structures having the highest concentrations of GABA and GAD. Oligodendrocytes accumulate [3H]GABA and are likely involved in GABA degradative activities.

The purpose of the present study is to examine the effects of disruption of the metabolism of GABA on the accumulation of iron in GABAergic projection sites. Gamma-vinyl GABA (GVG), an enzyme activated inhibitor of GABA-transaminase, was injected unilaterally into the globus pallidus and adjacent striatum or into the substantia nigra of the rat brain. Control animals received unilateral injections of saline into the same areas. Two days after injection the animals were perfused and 40 µm sections of the brain were processed with the Perls'+DAB histochemical method for iron. The intensity of iron stain was measured with densitometry.

Following GVG injection into the caudate putamen/globus pallidus there was a significant reduction in iron concentration in the homolateral ventral pallidum, globus pallidus and substantia nigra. There was no detectable difference measured in the nigrotectal projection sites when GVG was injected into the substantia nigra.

The results of this study provide evidence that the presence of iron in oligodendrocytes and in the neuropil is related to the metabolism of GABA.

286.8 BARBITURATE AND PICROTOXIN-SENSITIVE CHLORIDE EFFLUX IN RAT CEREBRAL CORTICAL SYNAPTONEUROSOMES. Rochelle D. Schwartz[1], Phil Skolnick[2]* and Steven M. Paul[1]*. [1]Clinical Neuroscience Branch, NIMH and Lab. Bioorganic Chem., NIADDKD, NIH[2], Bethesda, MD 20205.

GABA exerts its inhibitory action in brain by increasing membrane permeability to chloride ions (Cl^-). This phenomenon has been studied extensively using electrophysiologic techniques but because of methodologic limitations biochemical studies of Cl^- transport in cell free brain preparations have generally been unsuccessful. We now report $^{36}Cl^-$ transport in a novel subcellular preparation from rat brain, the "synaptoneurosome" (Hollingsworth et al., Fed. Proc. 43, 1093, 1984) that is sensitive to barbiturates and picrotoxin. Rat cerebral cortices were homogenized in a modified Krebs-Ringer buffer and filtered through nylon mesh and a 10 µm millipore filter. The filtrate was centrifuged at 1000 xg for 15 min. The supernatant was discarded and the pellet was resuspended in buffer at a protein concentration of 10 mg/ml and incubated with $^{36}Cl^-$ (5 uCi/ml) for 60 min at either 0° or 25°C. $^{36}Cl^-$ efflux was studied by diluting aliquots of $^{36}Cl^-$-loaded synaptoneurosomes 50 or 100 fold with buffer in the presence or absence of drug. At various time intervals the diluted suspension was filtered over Whatman GF/C filters which were then washed with cold buffer and counted in a liquid scintillation counter. A rapid efflux of $^{36}Cl^-$ was observed within the first 30 sec after dilution of the synaptoneurosomes. A slower phase of $^{36}Cl^-$ efflux followed. The convulsant, picrotoxin (1mM) significantly decreased $^{36}Cl^-$ efflux (26 ± 3% at 1 min) while pentobarbital (1mM) increased $^{36}Cl^-$ efflux (71 ± 13% at 1 min). The EC_{50}s for picrotoxin and pentobarbital were approximately 400 µM and 100 µM, respectively. The pentobarbital effect was inhibited by picrotoxin (1mM) in a competitive manner. Similar effects were observed in synaptoneurosomes prepared from cerebellum. The barbiturate effect was stereospecific since (-)dimethylbutyl barbituric acid (DMBB) was more potent than (+)DMBB at enhancing $^{36}Cl^-$ efflux. Barbiturates and picrotoxin are believed to act at a site on the Cl^- ionophore associated with the GABA receptor. Our results suggest that the synaptoneurosome preparation is a valuable tool for measuring physiologically relevant Cl^- transport associated with the GABA/Cl^- ionophore receptor complex.

286.9 $GABA_B$ RECEPTOR MODULATION OF CATECHOLAMINE-STIMULATED cAMP FORMATION IN RAT BRAIN: STRUCTURE-ACTIVITY REQUIREMENTS AND IN VIVO INTERACTIONS. E.W. Karbon*, R.S. Duman* and S.J. Enna. Depts. Pharmacol., Neurobiol. & Anat., Univ. Texas Med. Sch., Houston, TX 77025.

Studies have indicated that $GABA_B$ receptor agonists (i.e. baclofen) greatly amplify the production of cAMP in rat brain slices that occurs during exposure to norepinephrine (Karbon E.W., Duman, R.S. and Enna, S.J., Brain Res., in press). $GABA_A$ receptor agonists (i.e. THIP) are inactive in this regard, and the response is not blocked by bicuculline. To further characterize this phenomenon, the influence of a variety of GABA analogues on norepinephrine-stimulated cAMP accumulation in rat brain slices was examined using a cAMP prelabeling technique. For these studies the concentration of norepinephrine was held constant (100 µM) in the presence of various concentrations (0.1-1000 µM) of the GABA agonists. The relative potencies of these compounds in the cAMP assay were compared to their potencies as inhibitors of calcium-dependent ^3H-GABA binding in rat brain membranes, a measure of $GABA_B$ receptor sites. Both β-p-fluorophenyl GABA and β-phenyl GABA amplified the response to norepinephrine in the cAMP assay, but were less potent than baclofen in this regard. (R)-3-hydroxy GABA was found to be substantially more potent than the S-isomer in both the cAMP and $GABA_B$ binding assays. To test whether $GABA_B$ agonists amplify the response to norepinephrine in vivo, rats were treated chronically (5 days) with either baclofen alone (10 mg/kg, i.p., b.i.d.), imipramine alone (2.5 mg/kg, i.p., b.i.d.) or a combination of the two drugs. The animals were decapitated 18 hrs after the last drug treatment and norepinephrine-stimulated cAMP accumulation and β-adrenergic binding (^3H-DHA) studied in cerebral cortical tissue. Treatment with either drug alone had no significant effect on cAMP production, but a significant decrease in β-adrenergic binding and cAMP accumulation (30%) was observed following the drug combination. Since the rate of β-receptor desenitization is thought to be a function of receptor activity, these preliminary results suggest that baclofen amplifies the response to noradrenergic stimulation in vivo. In conclusion, these studies support the contention that GABA modulates the cAMP system in brain through an action at $GABA_B$ receptors, and indicate this effect may be a determinant in the receptor responses which occur following chronic treatment with imipramine. (Supported in part by grants from the National Science Foundation and Bristol-Myers Corp.).

286.10 INHIBITION OF MOUSE BRAIN GABA AMINOTRANSFERASE BY PHENYLTHIOHYDANTOIC ACID. Godfrey Tunnicliff* (SPON: H. Stanton). Laboratory of Neurochemistry, Indiana University School of Medicine, Evansville, Indiana 47712.

Evidence is accumulating that by increasing brain GABA levels, protection against seizures will ensue. In an attempt to discover compounds that can elevate GABA concentrations, a series of GABA structural analogues were tested as inhibitors of mouse brain GABA aminotransferase (GABA-T). Phenylthiohydantoic acid was the most potent of the 31 compounds tested. When partially purified GABA-T was assayed in the presence of inhibitor, a competitive inhibition was seen with GABA as the variable substrate (Ki=91 µM). At a fixed GABA concentration, a non-competitive inhibition was observed when the concentration of either α-ketoglutarate or pyridoxal phosphate was varied.

These results suggested that phenylthiohydantoic acid had the potential to inhibit GABA-T in the intact brain and thus to increase levels of GABA. Phenylthiohydantoic acid was then administered intraperitoneally to mice (3 mmole/kg) and the animals killed 2 hours later. The activity of GABA-T in whole brain homogenates was measured. Compared to untreated control animals, the activity of the enzyme was reduced 28% by the drug treatment.

These data strongly support the idea that phenylthiohydantoic acid can enter the brain from the blood stream and interfere with the catabolism of GABA. Further studies are required to establish if phenylthiohydantoic acid can act as an anticonvulsant agent.

286.11 REGIONAL AND SUBCELLULAR DISTRIBUTION OF PIPECOLIC ACID IN RODENT BRAIN. J.S. KIM*, E. GIACOBINI (SPON: C. Su), Dept. Pharmacology, Southern Illinois Univ. Sch. Med., Springfield, IL 62708.

The imino acid, pipecolic acid (piperidine-2-carboxylic acid, PA), represents the major product of lysine metabolism in mammalian brain (Giacobini et al., Cell. Mol. Biol., 26:135, 1980). A rapid and sensitive method for the quantitative determination of PA has been described (Kim and Giacobini, Neurochemical Res., in press). Quantification and identification of PA are accomplished using high performance liquid chromatography with electrochemical detection (HPLC-EC) and nipecotic acid (NPA), an isomer of PA, as an internal standard. The cyclic imino acids are derivatized with 2,4-dinitrofluorobenzene (DNFB) to dinitrophenyl derivatives. The total analysis time is less than 30 min and the limit of sensitivity is in the lower picomole range. The regional distribution of PA in rat and mouse brain is similar and higher concentrations (nmole/g) are seen in hypothalamus (rat, 4.1; mouse, 7.9), cerebellum (rat, 3.5; mouse, 8.1) and pons-medulla oblongata (rat, 4.0; mouse, 7.2). Cerebral cortex (rat, 1.7; mouse 3.4), olfactory bulb (rat, 1.9; mouse, 4.8), hippocampus (rat, 1.9; mouse, 4.8) and spinal cord (mouse, 2.6) showed lower concentrations. Levels of PA in mouse brain were about twice as high as in rat brain. Pipecolic acid levels were measured in subcellular fractions of mouse whole brain. Forty-one percent was found in the soluble fractions (S_2) and 24% in the crude mitochondrial fractions (P_2). The specific amounts of PA (pmole/mg prot) in brain homogenate, P_1, P_2 and S_2 were 29.3, 14.6, 32.3 and 76.6, respectively. These results suggest that PA can be accumulated in synaptosomes and, if released, exert a neuromodulatory action such as other neurotransmitter amino acids. The developmental levels of PA in mouse brain are higher (45.4 nmole/g) in early postnatal life and fall rapidly to adult levels (4.1 nmole/g) at two weeks after birth. This pattern is similar to that seen in amino acids such as proline and tyrosine, and suggests a specific role of PA in the perinatal period. (Supported by SIU Central Research Grant 2-40202 to E.G.)

286.12 EFFECTS OF GLUTAMATE RECEPTOR AGONISTS ON THE BASAL RELEASE OF ^{3}H-GABA FROM PRIMARY RAT CEREBELLAR CELL CULTURES. S.O. Lee and G.R. Dutton. Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

The effects of glutamate receptor agonists on the basal release of ^{3}H-GABA were studied in dispersed rat cerebellar cell cultures prepared from 7-9 day old animals grown 7-9 days in vitro (DIV). Quisqualate, kainate, and L-glutamate induced marked increases over the basal release of ^{3}H-GABA ranging from 400 to 650 %, whereas N-methyl-D,L-aspartate (NMDLA) had little effect. The rank order of potency was: quisqualate > kainate > L-glutamate > NMDLA. These increases were not antagonized by glutamate diethyl ester (GDEE), 2-amino-4-phosphonobutyric acid (APB), or γ-glutamylglycine. However, these increases were inhibited by increasing Mg^{2+}(15mM) and decreasing Ca^{2+} (0.1mM). GDEE and APB alone had no effect on the basal release of ^{3}H-GABA. Tetrodotoxin (TTX) was without effect on stimulated ^{3}H-GABA release when the cultures were treated with L-glutamate (50μM), kainate (50μM), or K^+ (50mM), indicating that action potentials were not involved.

At EC_{50}'s or at maximal stimulatory doses, simultaneous application of L-glutamate and kainate did not produce an additive stimulatory effect on ^{3}H-GABA release, suggesting that L-glutamate and kainate may act at the same receptor site. With the exception of quisqualate (2.5μM), cultures responded to multiple stimulation by K^+ (50mM), kainate (50μM), or L-glutamate (50μM), demonstrating the retention of viability of the cultures during the experimental procedure. Glial-enriched cultures preloaded with ^{3}H-GABA did not respond to L-glutamate or kainate stimulation, indicating that the enhanced release observed with the neuronal-enriched cultures was of neuronal origin. Therefore, the data show that quisqualate- and kainate-preferring glutamate receptor subtypes may be present and in greater abundance than the NMDLA-preferring receptor subtype in cell cultures prepared from postnatal rat cerebella.

This work was supported by USPHS grant NS 16518.

286.13 The Effect on GABA Receptors of Nipecotic Acid in Rabbit Retina is Maximal at Eye Opening. PAUL MADTES JR., Laboratory of Vision Research, NEI, NIH, Bethesda, MD 20205.

How the development of the GABA system is regulated and what factors influence its development has been the subject of many investigations. Previous study has demonstrated that a functioning uptake system for GABA is present at birth and that this uptake mechanism can be inhibited by in vivo treatment with nipecotic acid, a GABA analogue known to block GABA transport, resulting in a 4-fold increase in ^{3}H-muscimol binding. Subsequently, this phenomenon was shown by in vitro treatment to be mimicked by GABA agonists, indicating a direct involvement of the GABA receptor. The timecourse of the sensitivity of the postsynaptic membrane to this regulation is reported here. Isolated eyecups were prepared from rabbit pups at selected ages and incubated at 37°C for 45 min in an oxygenated Krebs- Ringers bicarbonate buffer with or without 10 mM nipecotic acid. The retinas were then removed, homogenized in sucrose, and stored frozen until assayed for ^{3}H-muscimol binding. It was found that the number of high-affinity sites was approximately 70% higher in the treated tissue, compared to control until about 9 days after birth, at which time the number of sites were 160% higher. After eye opening, the increase returned to about 13% above control levels. After maturity, the number of receptors decreased to 50% below control levels. The number of low-affinity sites was only stimulated at 3 days (80% higher), decreasing linearly to control levels by day 6 and were below control levels after eye opening. No changes were found in either high- or low-affinity K_d values for treated compared to control tissues. These findings lend support to the notion that GABA itself may be involved in the regulation of development and indicate that eye opening may represent a critical point in the maturation process of the GABA system in the retina.

286.14 THE RELEASE OF (^{3}H)-GABA FROM RABBIT RETINA. E. Agardh* and B. Bauer*. Department of Ophthalmology, University of Lund, Lund, Sweden.

The release of (^{3}H)-GABA from neurons and glia in the rabbit retina was studied. The results indicate that neither are there any glutamate, aspartate, or glycine receptors, nor any GABA autoreceptors on the GABA accumulating neurons.

We also report a Ca^{++}-independent, K^+-induced release of (^{3}H)-GABA from retinal neurons. 40 mM K^+ has been shown to release (^{3}H)-GABA from glia in rabbit brain, but we could not demonstrate any such effect on the glial cells in the retina. Ouabain has been proposed as a pharmacological tool to study transmitter release from a cytoplasmic pool, and caused an increased release of (^{3}H)-GABA from both neurons and glia. The actions of ouabain may be complex, but everything considered, it appears that the release of (^{3}H)-GABA from rabbit retina can be mediated also by mechanisms not involving synaptic vesicles.

287.1 INFLUENCE OF CHRONIC CHOLINE-CONTAINING DIETS ON NEUROBEHAVIORAL PARAMETERS IN THE C57BL MOUSE B. F. Mervis[1], L. A. Horrocks[2], L. J. Wallace[3], and E. Naber[4]. The Brain Aging and Neuronal Plasticity Research Group, Departments of Pathology[1], Physiological Chemistry[2], Pharmacology[3], and Poultry Science[4], The Ohio State University, Columbus, Ohio 43210.

Male C57Bl/6NNIA mice were chronically fed diets containing free choline or bound choline (as 95% purified phosphatidylcholine [PC] or as an oil-free commercial lecithin [Centrolex®]) for five months. The mice were placed on the diets when eight months-old and behaviorally tested (using a passive-avoidance paradigm) and sacrificed when 13 months-old. The choline in these diets--in free or bound forms--was enriched at low, medium, or high levels (containing 2.4, 4.8, or 10.8 mg/g of chow, respectively). Other diets contained lesser but adequate levels of free choline (0.9 and 1.5 mg/g) and a standard lab chow (Purina). All specially formulated diets were isocaloric and isonitrogenous.

When compared to lower levels of dietary choline, behavioral testing showed that supplementation with bound or free choline generally tended to significantly improve retention of learning. However, learning in these adult mice (who are already good performers) was not further significantly enhanced relative to the standard chow. Analysis of the lipid composition in a plasma membrane fraction from mouse forebrain (phosolipid composition, cholesterol content, total lipid phosophorous) showed that membrane composition remained remarkably constant despite the different long-term dietary regimens. However, indirect evidence of some enhanced membrane fluidity is suggested by a greater degree of fatty acyl unsaturation in PC-enriched dietary groups. Receptor binding studies on neocortex (muscarinic, α- and β-adrenergic), hippocampus (muscarinic, GABAnergic) and striatum (muscarinic) have indicated no clear-cut dose-response trends for any of these receptors as a consequence of any diets in this age range. (Supported by a grant from the Central Soya Company).

287.2 PROTECTION FROM NITROGEN-INDUCED AND POTASSIUM CYANIDE-INDUCED HYPOXIC LETHALITY ARE NOT DEPENDENT UPON BINDING TO THE ^{3}H-NITRENDIPINE RECEPTOR. R.L. Kochman, M. Nevins* and P. Sumner. Dept. of Biol. Res., G.D. Searle & Co., Skokie, IL 60077.

Hypoxic or ischemic attacks may cause the influx of extracellular calcium (Ca) into cell cytoplasm, resulting in a cascade of cytotoxic products that causes neuronal cell death or damage. We sought to determine if blockade of the slow voltage Ca channels would protect mice from the lethal effect of nitrogen (N_2) hypoxia or potassium cyanide (KCN) hypoxia.

Male Crl:CD-1(ICR)BR mice, 24-33 grams, were administered one of the test compounds intraperitoneally 30 minutes before either a 100 second exposure to 100% N_2 in an enclosed chamber, or an intravenous injection of KCN (3.2mg/kg). Control mice were administered vehicle (10ml/kg, ip). The mice surviving at each dosage of the test compound were counted. To estimate activity at the Ca channel, test compounds were evaluated in vitro for their ability to displace 0.3nM ^{3}H-nitrendipine (^{3}H-NT) from binding sites in mouse forebrain.

A number of different compounds protected mice from both N_2-induced and KCN-induced lethality: Ca channel blockers, diazepam, haloperidol, cinnanserin and pentobarbital. However, only the Ca channel blockers, haloperidol and cinnanserin displaced ^{3}H-NT binding. Indomethacin, pyritinol, ethanol and baclofen protected from the effects of KCN but not N_2, while physostigmine, DMSO and THIP protected mice from the effects of N_2 but not KCN. With the exception of ethanol, none of the compounds affected ^{3}H-NT binding. Piracetam, morphine, naloxone, aminophylline and other compounds neither blocked hypoxic lethality nor bound to the ^{3}H-NT receptor.

These preliminary experiments indicate that although Ca channel blockers protect mice from two kinds of hypoxia, binding to the ^{3}H-NT receptor is apparently not a prerequisite for this protection. In fact, the results imply that there may be several ways whereby different compounds protect from the effects of hypoxia.

287.3 ADAPTATION OF BRAIN TISSUE TO THE ACUTE EFFECTS OF ANOXIA: A STUDY USING THE RAT HIPPOCAMPAL SLICE PREPARATION. A. Schurr, K.H. Reid, M.T. Tseng*, C. West* and B.M. Rigor. Anesthesia and Critical Care Research Unit (ACCRU), School of Medicine, University of Louisville, Louisville, KY 40292.

While immature animals show a remarkable resistance to the acute effects of anoxia, adults exhibit much higher sensitivity to the same effects. It is not known with any certainty what factors are of primary importance in anoxic mortality in general, nor what factors are responsible for the increased ability of immature animals to survive anoxia. In addition to the many advantages of the hippocampal slice preparation as a model system, it allows separation of effects of anoxia on nervous tissue from those of ischemia. A dual, linear flow slice chamber was used in this study. Slices from hippocampi of mature rats (250-400g) were placed in both compartments of the dual chamber and maintained by perfusion with artificial CSF and a supply of 95% O_2/5% CO_2. CA1 population responses were evoked and recorded from one slice in each compartment by stimulating the Schaffer collaterals with a bipolar stimulating electrode. Upon changing the gas atmosphere from 95% O_2/5% CO_2 to 95% N_2/5% CO_2 the CA1 population response usually disappeared within 2 min. Most slices could tolerate 10 min of anoxia as a return to O_2 atmosphere usually brought back the CA1 response to its pre-anoxic amplitude. However they could not tolerate 15 min of anoxia. After pre-exposing one compartment of the dual chamber to a short (5-7 min) anoxic episode and allowing 30 min recovery period, the slices in this compartment were able to tolerate 13-15 min of anoxia while those in the other compartment could not. We hypothesize that the pre-exposure to an anoxic episode induces or activates a cellular mechanism, active in immature animals, which enables hippocampal neurons to survive future anoxic episodes. Electron micrographs show that the structural integrity of the adapted slices is better preserved than that of the non-adapted ones.

287.4 PLASTICITY IN THE SUPRAOPTIC DENDRITIC ZONE: DENDRITIC BUNDLE (BUT NOT DOUBLE SYNAPSE) FORMATION VARIES WITH CHRONIC DEHYDRATION. L.S. Perlmutter, C.D. Tweedle, and G.I. Hatton. Depts. of Psychology, Anatomy and Neuroscience Program, Michigan State Univ., East Lansing, MI 48824-1117.

Dynamic neuronal/glial interactions occur at all levels (soma, dendrite, axon) of the magnocellular neurosecretory cells (MNC) of the supraoptic nucleus. These cells increase production and secretion of oxytocin and/or vasopressin with dehydration, parturition, and lactation. Retraction of thin astrocytic processes results in increased neuronal membrane apposition and possibly in the formation of double synapses (one presynaptic terminal contacting 2 postsynaptic elements) with gestation and lactation (chronic stimuli). Only appositions vary with acute water deprivation (4-24h) at both the soma and dendrites. At the level of the soma, both measures vary with chronic dehydration and rehydration. Here we investigated dendritic zone response to chronic dehydration (1.5-2% saline instead of water for 10 days) and rehydration. 32 rats (4 females & 4 males/group) were used: Controls (tap water); 10 days saline; 10 days saline, 5 days tap water; 10 days saline, 14 days tap water. Thin sections of the dendritic zone were quantitatively analyzed (ANOVA, Newman-Keuls) at the ultrastructural level for dendritic bundle (2 or more dendrites with membrane in direct apposition) and double synapse formation. The mean number of dendrites per bundle varied with treatment ($p<.01$): Control± S.E.=1.36±0.05; 10 days saline=1.73±0.08, a 28% increase ($p<.01$). 14 days rehydration produced only a 7% decline (1.61±0.03,ns) in this measure. The percentage of dendrites contacted by double synapses did not vary with treatment, and there were no sex differences.

Dendritic bundling increased with chronic dehydration due to glial retraction, but did not return to control levels with rehydration as at the soma. Glia may modulate MNC activity in response to both chronic and acute stimuli by selectively altering the amount of neuronal membrane in direct apposition. Glial retraction may be a necessary but not sufficient condition for synaptogenesis, which so far has only been shown to occur in the dendritic zone with gestation. Some axo-dendritic double synapses are monoaminergic, while axo-somatic double synapses are not (Tweedle & Hatton, this meeting). Activation of one subpopulation of inputs during gestation but not dehydration is possible, suggesting a different role for the dendrites at parturition than during dehydration.

Supported by NIH grant NS 09140.

287.5 LONGTERM EFFECTS OF HYPOTHALAMIC LESIONS IN FEMALE RATS ON PLASMA LUTEINIZING HORMONE CONCENTRATIONS AND LH-RH NEURON MORPHOLOGY. C.P. Phelps and S. Saporta, Dept. of Anatomy, Univ. of South Florida Coll. Med., Tampa, FL 33612

Deafferentation of the hypothalamus constitutes a classical experimental approach to understanding neuroendocrine hypothalamic function. In most instances, evaluation of the effects of deafferentation on neuroendocrine function is made at one time interval after brain surgery. We have been studying the short and long term effects of deafferentation on luteinizing hormone (LH) release in the female rat and have observed functional recovery 4 mos after surgery. The magnitude and duration of pituitary release of luteinizing hormone (LH) after estradiol benzoate priming (EB, 5mg/rat) followed by progesterone (P, 2mg/rat given 48 hrs after EB) was studied in ovariectomized (OVX) rats. The design was to measure sequential LH surges before and up to 18 wks after retrochiasmatic frontal knife cut (FC) lesions which block ovulation. Included for each animal was EB+P induced LH surges before and again at 4, 8, 12, 16 and 18 wks after FC. Blood was removed at 10:00 hr just before EB (Ohr), again before P and also at 4, 5, 6, 7 and 8 hr after P. Plasma LH measurement was by RIA. Maximum increases (Δ max) in plasma LH in control OVX rats after EB+P treatment showed a 50% decrease over a 4 month period. As expected, rats given EB+P 4 weeks after FC had a 90% reduction in Δ max LH (92±73 ng/ml) when compared to preoperative levels (1072±242 ng/ml). Further EB+P treatment at monthly intervals revealed a gradual improvement in Δ max LH through 16 wks. However, at 18 wk post-FC there was a further 4-fold increase in Δ max LH so that FC and control rats were the same in their Δ max LH values. Immunocytochemical study of LH-RH neurons in the brains of FC animals revealed novel LH-RH-containing axons in relation to glial elements. LH-RH axons were seen within the FC glial scar tissue and coursing around fluid filled cyst perimeters when they occurred near cuts. Correlation of novel LH-RH neuron patterns seen in the rats 18 wks after FC with individual animal LH release patterns during the study provided evidence for a dynamic response to axotomy in parvicellular hypothalamic neurons. Thus, the LH-RH neuron system's variable response to experimental damage may contribute to the variations seen in individual animal plasma LH levels after EB+P given throughout the study.

Supported by a grant from the Whitehall Foundation.

287.6 HISTOCHEMICAL CHANGES IN THE MOUSE TRIGEMINAL SYSTEM AFTER SECTION OF THE INFRAORBITAL NERVE. V. Yip, W.-P. Zhang*, Q. Yan*, O.H. Lowry* and T.A. Woolsey. Departments of Pharmacology, Anatomy/Neurobiology and The Mc Donnell Center for Studies of Higher Brain Function, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The sensory projections from the whiskers of mice and other rodents have been shown to synapse somatotopically in three sub-nuclei in the brainstem trigeminal complex, the ventrobasal complex of the thalamus and the somatosensory cortex. Deafferentation of the whiskers in adult animals results in qualitative and quantitative changes in the metabolic activities of the posteromedial barrel subfield of the somatosensory cortex (PMBSF). [eg. Dietrich et al 1981.] The present study was to determine the time course and extent of similar changes, if any, in the subcortical trigeminal centers of the adult mouse after deafferentation. The right infraorbital nerve was sectioned in mice under surgical anesthesia and the animals allowed to survive for periods up to 26 weeks. Some brains were prepared for histochemical demonstration of the mitochondrial enzyme cytochrome oxidase (CO) and some were prepared for microchemical analysis of metabolic enzyme activities at various times postoperatively.

Decreases in CO staining intensity were observed in the brainstem ipsilateral to the lesion as early as one week after nerve section and in VB and the PMBSF later. Quantiative analyses of activities of oxidative enzymes such as malate dehydrogenase also showed time dependent decreases in the appropriate brain nuclei. The quantitative and qualitative comparisons of the histological and microchemical results at different times after the peripheral lesions may provide better understanding of the time course and extent of metabolic changes in the mouse trigeminal pathway following deafferentation.

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287.7 INHIBITORS OF POLYAMINES BIOSYNTHESIS AND BRAIN PLASTICITY V.A. Eterović, P.A. Ferchmin and J.G. Ortiz. Dept. Biochem., Sch. of Med, Univ. C. del Caribe, Cayey, P.R. 00634 and Dept. Pharmacol., Sch. of Med. U.P.R., San Juan, P.R.

In this work we test the hypothesis that polyamines are essential for the stimulation of brain growth by environmental enrichment. Inhibitors of putrescine synthesis or saline solution(S) were injected to rats exposed either to enriched condition (EC) or impoverished conditions (IC). In IC rats were kept in small, individual cages. In EC the animals spent 7 hours a day in large cages with littermates and objects to interact with; they spent the rest of the time in the small cages. Thus there were four experimental groups: S-EC, S-IC, Drug-EC and Drug-IC. The treatment started at weaning and lasted for 4 days. The brains were dissected into occipital cortex, remaining cortex, subcortex, and cerebellum plus medulla. RNA and DNA were done under blind conditions. Two inhibitors were tested: 1,3-diamino-2-hydroxypropane (DAHP, 90mg/Kg) was injected daily before exposure to EC, and α-difluoromethylornithine (DFMO, 200 mg/kg) which was injected before and after each daily exposure to EC. DFMO reduced body weights ($p<0.0005$) both in EC (10%) and IC (3.5%) rats, but did not affect brain weights. The EC did not affect significantly body weights, but increased occipital cortex ($p<0.05$) and total brain weight ($p<0.01$), in saline as well as in drug-treated animals. The increase in cortical RNA content was also seen in DFMO-EC vs. DFMO-IC and S-EC vs. S-IC groups ($p<0.001$). The RNA/DNA ratio was increased in S-EC vs. S-IC, but not in DFMO-EC vs. DFMO-IC. This lack of difference was due to an unexpected increase in the RNA/DNA ratio of the DFMO-IC vs. S-IC rats. Similar results were obtained with DAHP. It decreased body weights (4%, $p<0.05$) without affecting brain weights. EC increased total cortex weight ($p<0.025$) and total brain weight ($p<0.05$). RNA content was increased in both, DAHP-EC and S-EC vs. their respective IC littermates. The DNA content was reduced in both, EC and IC rats by DAHP ($p<0.05$). The RNA/DNA ratio was affected by the two main factors ($p<0.0001$) and their interaction ($p<0.025$). Summarizing, both inhibitors affected cortical RNA and DNA, but neither abolished the expected effects of EC. However, we cannot conclude yet that polyamines are not essential for the EC/IC effect, because, due to a complex regulatory mechanism, a decrease in putrescine is not necessarily followed by decreased spermidine and spermine. The final interpretation of these data must be postponed until polyamines are determined. (DFMO gift Merrell Internat. Strasbourg, France)

287.8 INCREASED CORTICAL THICKNESS IN MALE PROGENY FROM ENRICHED PARENTS BEFORE AND DURING GESTATION M.C. Diamond, D. Chui*, R.E. Johnson*, M. Chelgren,* E.R. Greer* and J. Gibbons.* Dept. of Physiology-Anatomy, Univ. California, Berkeley, CA 94720

Increments in cortical thickness occurred in brains of F_1, F_2 and F_3 generations of pups whose parents were environmentally enriched. Results of our 1971 experiment (Diamond et al., Int. J. Neurosci. 2:171) indicated that the body weights of newborn pups from the enriched parents were significantly greater than those from impoverished parents. Although cerebral cortical thickness was greater in F_1 pups from enriched parents than in those from impoverished parents, the differences were not significant. We now ask whether mature F_1 rats would show significant brain differences and whether effects of enrichment on brains from F_2 and F_3 generations would be evident?

The experimental design was as follows: At 60 days of age, 12 Long-Evans females were placed in an enriched condition; 12 Long-Evans females were housed in standard colony conditions. An identical design was used for the males. At 90 days of age, enriched males and females were separated 2 per cage for mating, as were the standard colony males and females. After five days the enriched and standard colony pregnant females were returned to their respective conditions until one day before parturition. At that time all pregnant females were placed in single cages where they lived with their offspring until weaning at 21 days of age. Then the F_1 animals lived three to a cage until 60 days of age when half were autopsied for cortical measurements; the other half were returned to the conditions of their parents. The basic design was repeated twice, producing F_2 and F_3 generations. When the F_3 pups reached 60 days of age, they were enriched for 30 days before autopsy.

Cortical thickness measurements were made from 20 micra-frozen-transverse sections in the frontal, somatosensory and occipital cortices of all rats. When comparing cortical thickness of the male rats in the F_1, F_2 and F_3 generations, there was a significant increase in thickness in some regions in successive generations. These results will be discussed.

287.9 HOMOLOGOUS EFFERENTS SPARED BY PARTIAL FIMBRIAL LESIONS CONTRIBUTE TO THE RECOVERY OF HIPPOCAMPAL CHOLINERGIC ENZYMES. A.R.Dravid and E.B. Van Deusen*. Preclinical Res. Sandoz Ltd. Basel, Switzerland; Dept.Dev.&Cell Biol.,Univ.Calif., Irvine, CA 92717.

Occurrence of post-lesion synapse replacement after sprouting of intact axons in the vicinity of a lesion (Physiol.Rev.61:684,1981) prompted us to investigate whether homotypic reinnervation could occur if homologous efferents innervating regions near to the denervated ones were spared by partial lesions of the septo-hippocampal pathway. In support of this postulate we observed a substantial recovery of enzyme markers for hippocampal cholinergic terminals after partial but not after complete fimbrial transection (Brain Res.277:169, 1983). We have also found that transection of medial or lateral halves of the fimbrial bundle resulted in region-specific enzyme reductions along the septo-temporal hippocampal axis, followed by significant recovery during the 8 week post-lesion period (Brain Res. submitted). The present studies indicate the role of spared fimbrial efferents in the recovery process.

Transection of the lateral half of the fimbrial bundle, sampling of the hippocampus into five septo-temporal regions and determination of ChAT and AChE activities were as described before (Brain Res. submitted). Lesioned rats were distributed at random into 3 groups. Groups I and II were sacrified at 1 and 9 weeks after lesioning, respectively. In group III rats, the remaining fimbrial fibres were severed (2nd lesion) 8 weeks after the first lesion and the animals sacrificed one week later. A comparison of ChAT and AChE activities in these three groups revealed that relative to the maximally depleted enzyme activities at 1 week (group I) the significant recovery observed in group II at 9 weeks was not apparent in group III animals. These findings thus demonstrate the essential role of fimbrial fibers spared by the partial lesions in the recovey of hippocampal cholinergic enzymes, probably representing replacement of degenerated cholinergic terminals.

287.10 CHANGES IN PRESYNAPTIC INPUT NUMBER TO RAT SUBMANDIBULAR GANGLION NEURONS FOLLOWING SALIVARY GLAND ATROPHY. M. D. Womble*, K. Vanderslice*, and S. Roper (SPON: S. Roper). Dept. of Anatomy, Univ. Colorado Health Sciences Ctr., Denver, CO 80262.

This work is part of a series of experiments designed to study how neuronal maturation parallels the development and maintenance of target tissues. Intracellular recordings from parasympathetic neurons of the adult rat submandibular ganglion were used to study how atrophy of the submandibular and sublingual salivary glands affected synaptic connections in the ganglion. Confirming prior investigators, each postganglionic neuron in adult animals received an average of 1.50 ± 0.60 (S.D.) preganglionic axonal inputs (N= 40 cells, 7 rats). Salivary gland atrophy was achieved by ligating the main excretory ducts. Three weeks after ligation, the wet weight was 24.7 ± 5.4% (submandibular) and 39.2 ± 11.6% (sublingual) of contralateral control glands. Furthermore, large changes in the ultrastructure of glandular acini, such as degranulation and cellular shrinkage, took place after ligating the salivary ducts. Ligations which did not directly interfere with the postganglionic axons innervating the glands did not cause any significant change in synaptic connections within the ganglion (1.52 ± 0.72 inputs/cell; N= 54 cells, 7 rats). When salivary ducts were ligated near the glands, postganglionic fibers to the glands were also damaged. Under these circumstances, our results indicate that there was a small, but significant increase in the number of preganglionic inputs per neuron (1.98 ± 0.95 inputs/cell; N= 44 cells, 6 rats). Experiments are in progress to determine whether this increase represents presynaptic sprouting or other possibilities. Thus, these data indicate that synaptic inputs to neurons in the submandibular ganglion may not be affected by striking changes in the glands which those neurons innervate, at least in the adult. That is, feedback regulations by target tissues onto their innervating neurons in this parasympathetic ganglion may not be as powerful an influence as it has been shown to be in other parts of the nervous system. Experiments are in progress to study the effects of salivary gland atrophy in neonatal rats and to determine whether major changes in the maturation of the target tissues will affect normally-occurring synapse elimination in the developing submandibular ganglion.

287.11 CHRONIC ETHANOL EXPOSURE ALTERS SYNAPTIC REORGANIZATION FOLLOWING PARTIAL DEAFFERENTATION OF RAT DENTATE GYRUS. D.W. Walker, M.A. King, R.L. Reep and B.E. Hunter. VA Medical Center, Dept. of Neuroscience and Alcohol Research Center, University of Florida, Gainesville, FL, 32610.

A rat model of the capacity for CNS plasticity after long term ethanol consumption was used to assess quantitatively the extent of reactive synaptogenesis in the dentate gyrus. Two groups of adult male Long-Evans rats were maintained on nutritionally complete ethanol- or sucrose-containing liquid diets for 20 wks. Ad lib lab chow and water were then given to the animals for 8 wks. prior to partial denervation of the dentate gyrus by unilateral electrolytic lesion of the entorhinal cortex. Forty days postlesion, horizontal 40 micron frozen brain sections were cut and alternate sections processed with the Timm's sulfide-silver or acetylcholinesterase (AChE) techniques. The bandwidth and optical densities of afferent terminal fields were measured in the dentate gyrus using a computer-based image analysis system (EyeCom II/DEC 11/23). Reorganization of commissural/associational (C/A) and septal afferents was evaluated from 10 x 10 arrays of measurements in each blade of the lesioned and unlesioned dentate gyrus of each animal. These 10 x 10 arrays of data consisted of measurements made automatically at 10 equally-spaced medial-lateral positions in each of 10 equally spaced horizontal sections.

Chronic ethanol alone produced a 7% shrinkage in the total width of the molecular layer of the buried blade as assessed by comparison of measurements on the side contralateral to the lesion in ethanol vs. control brains. Lesion alone produced an 11% shrinkage in total molecular layer width as indicated by comparing the lesioned and unlesioned sides in the control group. There was an interaction between the ethanol and lesion effects, since total width on the lesioned side was 19% less than that of the unlesioned side, in the ethanol group. In addition, the lesion-induced expansion in the bandwidth of the commissural/associational terminal field was 50% reduced as a result of prior chronic ethanol exposure.

These results indicate that chronic ethanol ingestion produces a residual alteration of the capacity for synaptic reorganization following denervating lesions.

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287.12 INFLUENCE OF CORTICOSTERONE ON NEURONAL PLASTICITY IN THE RAT HIPPOCAMPUS. S.F. Hoff[1],S.W. Scheff[2],K.Anderson[2]. Dept. Pharmacology, Chicago Med. School, N.Chicago, IL. 60064.[1] Dept. Anatomy, University of Kentucky, Lexington, KY 40536.[2]

To better understand the mechanisms underlying lesion-induced neuronal plasticity in the rat hippocampal formation, we have analyzed the effects of adrenalectomy and elevated corticosterone (CORT) on the initial time course of reactive synaptogenesis. Previously we have reported a delayed process of reinnervation in the dentate molecular layer (ML) in aged rats, which may be related to their elevated blood levels of CORT. In addition, elevated CORT levels inhibit collateral sprouting in young adults.

Male Sprague-Dawley rats (90 days old), which had received bilateral adrenalectomies, were given a unilateral entorhinal lesion (n=31). These animals were divided into two groups: (1) Adrenalectomized (ADX) animals (n=14) and (2) HICORT animals (n=17) which received a subcutaneous implant of a 200 mg CORT pellet. At 4, 10 or 30 days post-lesion, animals from each group were killed and prepared for electron microscopy. Normal and degenerating synaptic profiles were quantified on montages of the dentate ML, which was divided into thirds (inner, middle, outer). In all, 31,083 synaptic profiles were counted over 106,000 square microns of neuropil. Data from the above animals were compared against previously reported results for young adult and aged rats.

Reinnervation of the middle and outer thirds of the ML varied between groups in that the HICORT group demonstrated a reinnervation process very similar to young adults (26% synapse replacement by 10 days post-lesion). This is well ahead of aged rats ($p < .05$). ADX rats had a different response with 59% of all synapses replaced in the middle ML by 10 days post-lesion ($p < .05$ vs. young adult) and a response very similar to young adults in the outer ML. Degeneration clearance in the ADX group was equal to the young adults by 10 days post-lesion. The HICORT group had a slower response ($p < .05$ vs young adult), which was close to that of aged rats. Within the inner ML, both ADX and HICORT groups demonstrated similar non-degenerative changes in synaptic density, as we have reported in adult and aged rats. Previous studies have indicated that the rate of reinnervation may be related to the rate of degeneration clearance. Our present results suggest that this is not the case. Also, HICORT levels appear not to inhibit synapse reappearance, eventhough collateral sprouting into the middle ML is less. This may have functional consequences during CNS repair processes. (NIH grant 16981 to SWS).

287.13 EXPANSION OF REACTIVE GLIAL FIBERS IN THE NEUROPIL FOLLOWING DEAFFERENTATION. J. Wells and L.N. Tripp*. Dept. of Anatomy and Neurobiology, Univ. of Vermont, Burlington, VT 05405.

The glial reaction to deafferentation has been described often but rarely quantified. In order to study the temporal relationship of the glial reaction to the sequence of axonal growth and synaptogenesis, we have measured the area of the profiles of glial fibers in the neuropil of the thalamic ventral posterolateral nucleus (VPL) following lesions to its afferents. Partial deafferentation of VPL has been shown previously to produce reactive synaptogenesis (Brain Res. 155:362). In addition we have related the glial expansion to specific characteristics of the deafferentation: the size of the lesion, the degree of degeneration, the proximity of the lesion to VPL and the site of the lesion. To determine the time course of the glial expansion, suction lesions were made in the dorsal column nuclei (DCN). Other deafferenting lesions were: small heat lesions of the medial lemniscus at the pontomedullary junction, large heat lesions of the mesencephalon and suction lesions of the somatosensory cortex. Area measurements were made from electron micrographs of the VPL neuropil using morphometric procedures. Neuropil, as defined here, excludes the cell bodies of neurons and glia, myelinated fibers and blood vessels. Glial fiber profiles were identified using multiple criteria, e.g., irregular borders, fibrillar arrays and glycogen granules.

Glial fibers occupied 9.8μm^2/100μm^2 of the normal VPL neuropil. Following DCN lesions the area of glial fibers doubled within 24-48 hours. Many of the expanded profiles are seen in association with degenerating terminals. After 4 and 6 days the expansion was reduced but still was 50% greater than controls. At 30 days the area of glial fibers was significanlty less than controls and it was at this time when synaptogenesis began in this system. The glial processes have returned to normal by 50 days and synaptogenesis was also complete.

When VPL was deafferented by lesions of the medial lemniscus, mesencephalon or somatosensory cortex, the increased area of glial fibers was comparable to that after DCN lesions. The results further indicate that the amount of glial expansion was independent of the following: the size of the DCN lesion (r=0.208), the degree of degeneration in VPL (r=0.131), the proximity of the lesion to VPL, or the site of the lesion (i.e., the type of afferent destroyed). Thus, the response of the glial fibers was relatively constant regardless of the characteristics of the lesion.

287.14 **CHANGES IN DENTATE GYRUS LAMINAR PROFILES FOLLOWING ENTORHINAL CORTEX REMOVAL IN THE AGED RAT.** J. Vicedomini*, S.W. Scheff, S.T. DeKosky (SPON: A. Nonneman). Depts. Anatomy and Neurology, Univ. of Kentucky and V.A. Medical Center, Lexington, KY 40536.

Unilateral entorhinal cortex removal selectively denervates the outer molecular layer in the ipsilateral dentate gyrus. Axonal sprouting, induced by entorhinal cortex removal, results in circuitry alteration of residual afferent systems and synaptic replacement through a process known as reactive synaptogenesis. Septal, commissural/associational, and crossed tempro-ammonic afferents are partially responsible for reinnervation of the deafferented zone. This reactive process follows a well defined time course in the young adult rat. The rate of septal and commissural/associational sprouting is reduced in aged relative to young adult rats. Little is known concerning the effects of advanced age on sprouting in the crossed tempro-ammonic pathway.

We have electrophysiologically monitored the time course of crossed tempro-ammonic sprouting in young (3 month), middle age (16 month) and aged (26 month) F344 rats. At each age level different groups of subjects were examined 15, 30, 45, and 60 days after entorhinal cortex removal. Extracellular field potentials, evoked by stimulation of the intact entorhinal cortex, were examined in the ipsilateral and contralateral dentate gyrus. Laminar profile analyses were conducted to reveal the extent of functional synaptic replacement. Input/output relationships at different stimulus intensities were plotted to compare the efficacy of sprouted crossed tempro-ammonic afferents with that of the intact ipsilateral perforant pathway.

Laminar profile analyses indicated that even in the aged animals the crossed entorhinal projection expands its affernt projection and makes functional contacts in the denervated zone. In all three age groups the altered circuitry was capable of supporting population spike activity however with a delayed time course in the aged animals. These results indicate that the lesion-induced altered circuity is capable of restoring some of the electrophysiological properties in the aged CNS. (Supported by NIH grants NS16981, NS00444, 5T32AG00084, BSRG S07R05374 and the VA Medical Research Service).

287.15 COMPENSATION OF PARALLEL FIBER-PRESYNAPTIC GRIDS FOLLOWING DEFICITS IN NUMBER OF SYNAPSES ON PURKINJE CELLS. S. Chen and D. E. Hillman, Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Recent results show that total contact area of postsynaptic membrane densities (PoSDs) was constant following a wide range of reductions in the number of parallel fiber synapses on Purkinje cells (Brain Res. 295:325-343, 1984). Deficits in the number of afferent sites from development or arising in adulthood resulted in an increase in total area of presynaptic contact made by each afferent neuron until a 200% level was reached (Soc. Neurosci. Abst 9: 1224, 1983). Here, we demonstrate that the total number of dense projections of the presynaptic grid increases on each axon of remaining granule cells.

The number of dense projections and area of the presynaptic grids were determined for comparisons between control females and groups of rats with previously determined reductions in the number of synapses on Purkinje cells. This reduction was induced by either developmental protein restriction or lesions to granule cells in the adult. The animals were prepared by glutar-paraformaldehyde perfusion and the tissue was stained with ethanolic-phosphotungstic acid. Completeness of presynaptic grids in sections was assured by serial reconstruction of enface sites. The number of dense projections and the area of each site were recorded and compared by plotting a linear regression line for the composite data along with a standard line for constant density of dense projections.

The synapses in controls rarely had over 40 dense projections. A significant percentage of the experimental animals and especially those with severe deficits in number of synapses had 40 to 100 dense projections. Comparison of regression lines shows that the density of dense projections is constant even though the total area of afferent synapses is increased. These results demonstrate that afferent neurons compensate the loss of their kind by adding dense projections onto existing presynaptic sites rather than spreading of the presynaptic membrane specializations. The efficacy of enlarged sites is suggested by a larger area for synaptic vesicle attachment during release giving rise to a greater chance for postsynaptic events. Supported by Grant NS13742 from NINCDS.

287.16 NEURONS IN THE EAR MIGRATE AND SHIFT CONNECTIONS THROUGHOUT POSTEMBRYONIC LIFE. J.T. CORWIN. Department of Zoology and Bekesy Laboratory of Neurobiology, University of Hawaii, Honolulu, HI 96822.

Determinant growth patterns common to birds and mammals appear to be linked with finite periods for production of sensory and neuronal cells. However, most vertebrates grow indeterminantly, continually enlarging their sensory organs and brains through production and accumulation of new cells.

In fish and amphibians, auditory hair cell populations increase up to 12-fold through production that continues for years after birth. Microautoradiography has demonstrated that hair cell production occurs primarily at the periphery of epithelia, beneath hair cells that have small cilia. Electron microscopy has demonstrated that early embryonic ears contain exclusively the same small-cilia type of hair cell. Thus, postembryonic growth results from a continual accumulation of new hair cells at the edge of the epithelium (Corwin, 1984, Assoc. Res. Otolaryngol. Abstr. 7:56).

In the macula neglecta auditory epithelium in skates hair cell number increases postembryonically from 500 to more than 6000, but the innervating neurons do not increase in number (Corwin, 1983, J. Comp. Neurol. 217:345). Growth of the terminal arborizations of these neurons has been investigated here in macula neglecta epithelia from a size/age series of the skate, Raja ocellata. Distributions of nearest-neighbor distances for all terminal arbors in maculae from 2- and 5-year-old skates have similar shapes, but mean distance doubles by 5 years. Growth results in an even expansion of space between arbors, with most migrating out from the central epithelium that contains the oldest hair cells that first received all the innervation. The mean arbor area increases from 2800 to 8500 μm^2 per neuron, and the number of hair cells enclosed increases from 40 to 120 per neuron, but the proportional area of the whole epithelium enclosed remains at an average 4% per neuron over the 2- to 6-year age range. Orientation measures for 1738 branches from 103 neurons demonstrated that 82% of the branches grow toward the epithelium edge, with the angles in a normal distribution perpendicular to the edge.

This evidence demonstrates that synapse shifting and terminal arbor migration can persist through life in nervous systems that incorporate new cells. It also suggests that new hair cells that are not innervated may attract growing auditory neurons toward the epithelium edge.
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288.1 NEUROVASCULAR, AND NEURONAL ALTERATIONS IN FETAL HYPOTHALAMIC HETEROGRAFTS STEREOTAXICALLY POSITIONED IN THE THIRD CEREBRAL VENTRICLE OF BRATTLEBORO RATS. D. E. Scott and D. Sherman*. Dept. of Anatomy, Univ. of Missouri-Columbia, Columbia, MO. 65212

Immunocytochemistry, microangiography and transmission electron microscopy have been used to assess the anatomical alterations in the neuronal and neurovascular organization of fetal hypothalamic explants, 17 days post coitus, introduced into the third cerebral ventricle of 8 Brattleboro rats. Techniques for stereotaxic surgery and technical processing have been described elsewhere, (Gash and Scott, 1980; Scott et al., 1984). Tissue from rats killed 3, 6, 9 or 12 days after surgery was compared to tissue removed 30, 60 or 90 days post-surgery in an earlier study. Fine delicate vascular varicosities were observed in the 3 day fetal explants. It is yet to be determined whether they are intrinsic or extrinsic. Vascular networks of the surrounding host ventricular wall and periventricular stratum were larger in diameter and more profuse. By 9 days fetal hypothalamic explants were well vascularized. Major vascular invasion arose from host portal capillaries in the underlying median eminence, as well as lateral invasion from the periventricular wall of the host brain. By 30 days, vascularization appeared complete with vessels invading from the entire periphery around the third cerebral ventricle. At the ultrastructural level, neurites were seen to actively organize around the perivascular space of fenestrated vessels in the ventral portion of explants. Axon profiles, containing numerous dense core vesicles and terminating on the abluminal basal lamina of well developed perivascular spaces, were seen throughout the ventral third of the explants. Fragments of 17 day pc. fetal cortex employed as controls actively grew and flourished in the third cerebral ventricle of Brattleboro hosts. Neuritic, ependymal and vascular processes from host ventricular wall grew across the intervening ventricular lumen and were seen to insinuate into the parenchyma of the fetal heterograft. This suggests the presence of vaso- and chemotaxic agents within the explant. From these data it is clear that vascularization of fetal explants is rapid, and that the anatomical substrate(s) for the central delivery of neuropeptide hormones are established in viable fetal explants introduced into the brains of Brattleboro rats. Supported by NSPHS Grant NS19197.

288.2 ELECTRON MICROSCOPY OF RAT EMBRYONIC NEOCORTEX TRANSPLANTED INTO ADULT RAT SPINAL CORD. R. Trachimowicz and B.H. Hallas Department of Anatomy, New York College of Osteopathic Medicine, Old Westbury, NY 11568.

In previous cytologic and degeneration studies Hallas demonstrated that rat embryonic neocortex transplanted into adult rat spinal cord not only developed into cerebral cortical tissue resembling normal cerebral cortex with a laminar organization and fully differentiated stellate and pyramidal cells, but also established afferent and efferent connections with the host central nervous system (Experentia 38:699-701, 1982; Neurosci. Abst. 1983). Until now, however, the ultrastructure of the cortical transplants, including their synaptology, has not been described.

The present study was undertaken to provide a morphologic description and determination of the location, on the cells, of both afferent and intra-transplant synapses of the cortical transplants. Using the transplantation techniques of Hallas (1982) 15 day old rat embryonic neocortex obtained from pregnant Sprague-Dawley albino rats was transplanted into the spinal cord of adult Sprague-Dawley albino rat hosts between vertebrae C5 and C6. The transplants were allowed to grow, differentiate and establish connections for 150 days at which time the animals were sacrificed by transcardial perfusion with 3% glutaraldehyde in cacodylate buffer (pH 7.2). The segment of the cord containing the transplant was dissected out, cut into 1-2 mm cross sections and fixed an additional hour. Subsequently the tissues were postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethyl alcohols and propylene oxide and embedded in Araldite. Ultrathin sections of the transplant and associated cord were cut, mounted on grids, stained with uranyl acetate and lead citrate and examined with an RCA electron microscope. A comparison of the synaptology of the cerebral cortical transplants and normal adult cerebral cortex is described.

288.3 DENDRITIC AND AXONAL MORPHOLOGY OF FETAL NEOSTRIATAL CELL SUSPENSIONS IMPLANTED INTO ADULT NEOSTRIATA. J.P.McAllister, M.A.Reynolds*, L.Kaplan* and P.D.Walker*. Dept. of Anatomy, Temple Univ. School of Medicine, Philadelphia, PA 19140.

We have demonstrated that dissociated fetal neostriatal neurons will survive when transplanted to adult neostriata that had been lesioned with ibotenic acid (McAllister, J.P., Anat. Rec., 3: 107A, 1983). The present study sought to determine if these transplants (1) exhibit normal neuronal morphology and (2) receive connections from the host. Host rats received single unilateral injections of ibotenic acid placed stereotaxically into the neostriatum at 6 weeks of age. Five days later, striatal ridges were dissected from 14 day old fetuses and dissociated in a 0.1% solution of trypsin. Five microliters of this cell suspension was injected into the host neostriatum. After a 4-6 week survival period the host neostriata were processed by Rapid Golgi methods. Most of the neuron types that populate the normal neostriatum were identified in the transplant as early as 34 days post-transplantation. Spiny I neurons were most prevalent, with medium-sized somata, abundant spines on distal dendrites and a rich plexus of axon collaterals. Spiny II neurons, with fewer spines, were seen less frequently, and were limited to those with medium-sized somata. Both types of Spiny neurons exhibited long axons, but these were never seen leaving the transplant. Several Aspiny III neurons were identified with sparse dendritic processes and varicosities. No large Aspiny II or medium Aspiny I neurons were observed in any transplant, but an occasional Neuroglia form cell was noted. Although there were no clear examples of axons crossing the host-transplant interface, several types of afferent fibers were present within the transplant. The most abundant axons were thin with a rich pattern of collateral branches and beaded terminals, and thus resembled catecholaminergic afferents. A thick plexus of these fibers was usually observed in the caudal poles of transplants, while the rostral poles contained only patches. Thick axons with varicosities were seen coursing for some distance through the transplants. They gave off branches at acute angles and are thought to originate in the diencephalon or brainstem. A few extremely thin axons were observed with a long, straight main shaft and numerous side twigs. These may be cortical in origin. These data indicate that dissociated fetal cells from the striatal ridge can differentiate into mature-looking neurons within 4 to 6 weeks and that the transplants may be innervated by extrinsic afferents of the host. (Supported by BRSG #SO7RR05417)

288.4 RECOVERY OF FUNCTION AFTER HIPPOCAMPAL DAMAGE : EFFECTS OF EMBRYONIC C.N.S. TRANSPLANTS AND POST OPERATIVE ENVIRONMENT C.KELCHE (1), D.G.STEIN(2) and B.WILL (1). (1) Lab.de Neurobiologie des Comportements, Université Louis Pasteur 7, rue de l'Université 67000 Strasbourg. France. (2) Department of Psychology, Clark University. 950 Main street Worcester Massachusetts 01610. U.S.A.

The aim of this study was to examine whether a better recovery of behavioural functions after hippocampal damage is promoted by a combinaison of post-operative "enriched" environment and an embryonic hippocampal C.N.S transplant. One group of 10 adult male rats was sham-operated and four groups of 10 rats received bilateral dorsal hippocampal lesions by suction. One week later, the four groups with lesions received either 1) an hippocampal implant (two groups) , 2) a frontal cortex implant or 3) non implant. The implants were taken from 16 to 18 day-old embryos. Two days after implantation, one of the two groups with hippocampal transplants was placed in an "enriched" environment; the other four groups were placed in standard social conditions (same number of animals in similar cages as for the "enriched" condition, but without objects). The rats were tested for spatial memory in an 8-arm radial maze and for activity in a closed-field arena after 60, 120 and 180 days of differential housing. In the radial maze task, sham-operated rats made significantly fewer errors and were significantly less active in the closed-field test than were rats with lesions whether or not the latter had implants. The implants, whether specific (hippocampus) or non specific (frontal cortex), had no significant effect on the behaviour of lesioned rats reared in a social condition in the radial maze task. However, an unexpected result was that the group of hippocampectomized rats that had been implanted with embryonic hippocampal tissue and placed post-operatively in an "enriched" environment, showed an even higher activity level than that already evident in hippocampectomized rats that received no implant. Similarly rats implanted with embryonic hippocampal tissue and housed in a standard condition, showed a tendency to be more active than rats with hippocampal lesions alone.

The interaction of an "enriched" environment and embryonic hippocampal implants on the recovery of behavioural function after bilateral hippocampal lesions in adult rats is discussed in the light of anatomical and histological analysis (H.R.P method) of the grafted brains.

288.5 LONG-TERM SURVIVAL OF THE EMBRYONIC HYPOTHALAMUS TRANSPLANTED TO KIDNEY CAPSULE. J. Schechter*[1], N. Ahmad*[1], D.M. Gash[2], and M. Gupta[2,3]. Dept. of Anatomy and Cell Biology[1]. University of Southern California, LA, California, and Dept. of Anatomy[2] and OB/GYN[3], University of Rochester School of Medicine, Rochester, NY, 14642. (Sponsored by Anne S. Kaplan)

Our previous studies have shown that the hypothalamic anlage from 12 day old rat embryos can undergo morphological differentiation along the lines of normal development in an ectopic site such as beneath the kidney capsule (Schechter et al. Cell Tiss. Res. 190:247,1978). The present study was conducted to extend these observations and determine if the grafted presumptive hypothalamus could survive for prolonged periods beneath the kidney capsule and if the grafted tissue was responsive to the hormonal milieu of the host. Wistar Lewis rats (an inbred albino strain) were used as donors and hosts thus eliminating the possibility of graft rejection. The anlage of the ventral hypothalamus and neural lobe along with Rathke's pouch (anlage of the adenohypophysis) were grafted beneath the kidney capsule of adult host females. The host animals were either 1) intact, 2) hypophysectomized, or 3) ovariectomized, adrenalectomized and treated with estradiol benzoate. Grafts were allowed to develop for up to 180 days and then were recovered for light and electron microscopy.

Neural tissue developed with a high degree of organization with neurons and glial cells, and a large number of dendritic and axonal processes in all three test groups. The neurons varied in size, with large nuclei, ribosomes, rough endoplasmic reticulum, a well developed Golgi apparatus, and a large number of neurosecretory granules. Synapses were plentiful ranging from rather well-developed contacts to those with minimal specializations.

The present study has shown that the anlage of the hypothalamus survives for up to 180 days under the kidney capsule of an adult host, retains its cytoarchitectural integrity at the light microscopic level and exhibits normal ultrastructural features. An analysis of the influence of the hormonal milieu upon the ultrastructural features is now being conducted. It is clear that a long term survival of neural tissue in sites ectopic to the central nervous system will permit unique experiments that are not possible in the intact brain or in tissue culture.

Supported by NIH CA 21426 to J.S.

288.6 SURVIVAL OF INTRAHIPPOCAMPAL TRANSPLANTS OF CHOLINERGIC NEUROBLASTOMA INTO SEPTAL LESIONED RATS. J.H. Kordower, M.F.D. Notter and D.M. Gash. Dept. of Anatomy, Univ. Rochester Sch. of Med., Rochester, N.Y. 14642.

The technique of neural transplantation has received significant attention in recent years. Many paradigms have used either fetal tissue or peripheral ganglia as donor material to assess both anatomical and behavioral functionality following genetic or experimental lesions. Amitotic neuroblastoma cells may also be a useful source of donor material since 1) certain cell lines produce specific neurotransmitters, thus allowing greater precision in investigating animal models of disease states with specific transmitter abnormalities; 2) A pre-determined optimal number of cells can be transplanted; and 3) these cells can be maintained in culture indefinitely, adding greater flexibility in experimentation.

Long Evans rats received medial septal lesions to remove cholinergic afferents to the hippocampus. Five days later they underwent implantation of either C1300, IMR-32, or LA-N-2 (a gift from Dr. R. Seeger), cholinergic neuroblastoma cell lines into the left hippocampus via two 1 ul injections of approximately 10,000 cells each. Half of rats receiving C1300 or LA-N-2 cell transplants received cells that were treated with prostaglandin-E_1-cAMP to induce differentiation and prevent mitosis. The C1300 cells are derived from a mouse tumor, while the IMR-32 and LA-N-2 cells are a human neuroblastoma cell line. Rats were sacrificed 3 or 7 days after transplantation, their brains sliced in 5μ paraffin sections, and examined.

For all cell types, neuroblastoma cells were visualized along the cannula tract and in hippocampal areas distal to the cannula tract. The undifferentiated cells maintained a tumorous state, and by day 7 had invaded the entire hippocampus. These cells contained mitotic spindles and were seen in various stages of mitosis. The differentiated cells remained amitotic and appeared to migrate along the mediolateral plane of the hippocampus. The cell lines displayed different morphologies, with the LA-N-2 cells being largest and containing the most cytoplasm and the C1300 cells being the smallest with much less cytoplasm. Many cells in each experimental condition appeared adjacent to the blood vessels of the host. While lymphocytes were not present, a small number of neurofils were seen in both the differentiated and undifferentiated transplants.

This data provides preliminary evidence for the survival of cholinergic neuroblastoma cells in a cholinergic denervated brain site suggesting its use as donor material for neural transplants.

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288.7 VASOPRESSIN NEURONS GRAFTED INTO ADULT NEUROHYPOPHYSECTOMIZED HOSTS: STRUCTURAL AND FUNCTIONAL CORRELATES. F.F. Marciano*, D.C.D. Rohrer* and D.M. Gash (SPON: B. Weiss). Department of Anatomy, University of Rochester, Rochester, NY 14642.

Our laboratory group has shown that fetal neurons containing the neuropeptide vasopressin can survive transplantation into the brain of adult Brattleboro rats and function appropriately by alleviating the symptoms of diabetes insipidus within the recipient. Only grafts which met the following set of anatomical and immunohistochemical criteria have been shown to be effective in reversing the physiologic consequences of vasopressin deficiency; (1) the graft must be closely juxtaposed to the host median eminence, (2) contain well-differentiated vasopressin magnocellular neurons, (3) fibers from the above neurons must project into the well vascularized neurohypophysial region so that the neuropeptide can be released into portal circulation. To further test this neural transplant model system, a series of grafts were stereotaxically placed into adult neurohypophysectomized male Long Evans rats.

The animals used in this study were housed in metabolism cages with water consumption, urine osmolality and urine volume measurements being taken for two weeks prior to and 40 days after transplantation. Anterior hypothalmi, including the supraoptic nucleus containing vasopressin neurons, were microdissected out from 17 day Long Evans fetuses and injected into the 3rd ventricle of adult male Long Evans rats. Control animals were also neurohypoxed and received a sham transplant that consisted of only sterile culture media. Neurohypophysectomy gives rise to a transient diabetes insipidus as a result of the retrograde degeneration of neurons in the supraoptic and paraventricular nuclei and the removal of pituitary stores of vasopressin.

Operated animals which received transplants and met the above stated criteria for functionality (n=8) produced more concentrated urine ($p < .01$) and consumed less water ($p < .05$) than control animals (n=8) or operated animals whose grafts failed to meet the criteria (n=5). In fact, grafts that failed to meet the anatomical criteria for functionality showed an increased diabetes insipidus ($p < .01$) as compared to controls. Thus failure to establish appropriate connections with the host brain seemed to exacerbate the polydipsia and polyuria.

The results of this study suggest that structural integration with the appropriate target tissue in the host brain is important for the antidiuretic properties of this neuropeptide to be observed. Supported by NIH grant NS 15109 (DMG).

288.8 PROPERTIES OF PRIMATE ADRENAL MEDULLA CELLS IN VITRO AND TRANSPLANTED INTO THE CORTEX OF AFRICAN GREEN MONKEYS. D.M. Gash, M.F.D. Notter, A.L. Kraus*, S.H. Okawara*, J.J. Lopez-Lozano, and S. Wechkin[1]. University of Rochester School of Medicine and Dentistry, Rochester, NY 14642 and [1]SUNY, College at Brockport, Brockport, NY 14420.

It is well established that neural transplants in rodent model systems become structurally and functionally integrated into the host nervous system. The ability of transplanted neurons to correct CNS deficiencies has extraordinary theoretical and clinical implications. An example of the latter is the postulated use of dopamine-producing tissues grafted into the striatum of patients suffering from Parkinson's disease. It would seem that a key step in determining the clinical feasibility of using neural transplants to alleviate neural dysfunctions is to demonstrate that these grafts are safe and effective in non-human primates. Our research group is now investigating the development and function of neural and neural-like tissues into African Green Monkeys (Cercopithecus aethiops).

The present communication describes our studies on the short-term survival (2-3 weeks) of primate adrenal medulla tissue both in culture and transplanted into cortical cavities in C. aethiops. The adrenal medulla was recovered surgically from a 3 month old juvenile male and a 7 year old adult female monkey. Adrenal medulla cells placed in culture (media consisted of Eagle's minimum essential medium + .6% glucose, 20% fetal calf serum and 500 ng NGF/ml) survived for the three week test interval. The cultured cells lost their glandular appearance and assumed a more neuronal phenotype with angular perikarya and long axonal-like neurites. Glyoxylic acid histofluorescence and tyrosine hydroxylase immunocytochemistry confirmed that the cultured cells contained catecholamines including dopamine.

The survival of transplanted blocks of adrenal medulla cells into transplantation cavities in the cerebral cortex of adult hosts was also examined. Three weeks after transplantation, large numbers of surviving cells were identified clustered in cords resembling those seen in the intact adrenal medulla. These cells were glandular in appearance and exhibited the histochemical staining properties of the normal adrenal medulla. Thus, in culture in the presence of NGF, adrenal medulla cells assume a neuronal phenotype while transplanted in the host brain our initial studies indicate that they retain their glandular appearance.

Supported by the Brain Fund, University of Rochester Medical Center.

288.9 DEVELOPMENT OF EMBRYONIC NEOCORTICAL TISSUE TRANSPLANTED INTO THE CEREBELLUM, FOREBRAIN AND SPINAL CORD OF ADULT HOST ANIMALS. B.H. Hallas, New York College of Osteopathic Medicine, Old Westbury, New York.

Fifteen-day embryonic rat neocortical tissue was transplanted into either the cerebellum, forebrain, or spinal cord of 150 day old adult rat hosts. Host animals were sacrificed 6 hours, 1 day (d), 2d, 3d, 4d, 5d, 8d, 15d, 30d, 45d, 60d, and 150 days post-transplantation by transcardial perfusion with 10% neutral buffered formalin. The blocks of brain containing the transplants were embedded in paraffin, serial sections were cut, and alternate sections were stained with either cresyl-violet, Luxol-fast-blue, Bodian or a modified Weigart stain. Additional host animals were sacrificed and the transplants processed for Golgi-Cox impregnation, embedded in celloidin and serially sectioned.

All neocortical transplants survived, grew, differentiated and contained stellate and pyramidal cells that mimicked their in vivo counterparts. In addition, at the interface between transplant and host brain in no host animal was there a neural glial scar formation observed isolating the transplant from the host brain. All transplants had become integrated with the surrounding host neural tissue. However, there were differences in the growth and differentiation patterns of the neocortical tissue when transplanted in different regions of the host central nervous system in surviving numbers of neurons, and in the ultimate size of the transplant.

288.10 TRANSPLANTS OF FETAL BRAIN TISSUE CAN RESTORE BRIGHTNESS DISCRIMINATION ABILITY IN ADULT RATS WITH LESIONS OF THE OCCIPITAL CORTEX. Donald G. Stein, Randy Labbe,* Michael J. Attella,* Holly Rakowski* and Arthur C. Firl.* (SPON: D. Chad). Clark University Brain Research Laboratory and Department of Neurology, University of Massachusetts Medical Center, Worcester, MA 10610.

We have previously shown that fetal brain tissue transplants can partially restore spatial alternation learning in adult rats with bilateral lesions of the frontal cortex. The present experiments were designed to examine the question of whether fetal brain tissue implants could also overcome some of the visual deficits that often accompany bilateral lesions of the occipital cortex.

Accordingly, we created occipital cortex lesions by aspiration and 7 days after this surgery implanted, directly into the wound area, embryonic visual cortex or embryonic frontal cortex taken from rats in their 19th day of gestation. The animals with cortical tissue implants were then compared on a series of brightness and pattern discrimination tasks to rats with occipital cortex lesions alone or to intact counterparts tested in the same apparatus. We found that all three lesion groups were impaired on a pattern discrimination task. However, rats with implants of fetal frontal cortex were able to learn the brightness discrimination significantly faster than those with either lesions alone ($p<.05$) or implants of fetal occipital cortex ($p<.05$). In contrast, the rats who had received implants of fetal occipital cortex were as impaired as those with the occipital cortex lesions alone.

These unusual findings may have been due to the possibility that occipital cortex is more mature at E19 than the frontal cortex. If this were the case, the occipital tissue would release less neurotrophic factors into the wound areas than the less mature frontal tissue. In any case, the specificity of homologous tissue transplants is clearly not necessary for the transplants to mediate functional recovery from C.N.S. lesions.

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288.11 **BEHAVIORAL AND ANATOMICAL ASPECTS OF WHOLE ADRENAL GLAND TRANSPLANT INTO NEONATAL RAT BRAIN.** J. P. Kesslak, V. R. Holets, P. C. Mazur* and C. W. Cotman. Dept. of Psychobio., Univ. of Calif., Irvine, CA 92717.

Previous studies have demonstrated that adrenal chromaffin cells transplanted into brain may develop neural processes (Nature, 1981, 292, 351), but glucocorticoids can inhibit formation of these processes in vitro (PNAS, 1978, 75, 3498). We were interested in determining how whole adrenals transplanted into brain developed anatomically and if transplants could compensate behaviorally for loss of adrenals glands. A small cavity was made in the entorhinal cortex of 4 day old Sprague-Dawley rats. Embryonic (E16-18) adrenals were placed in the host four days later. Approximately 30 days after transplantion, adrenal glands were removed from implanted (IMP-ADX) and a group of nonimplanted (ADX) rats. Control animals did not receive any surgical treatment.

Rats were tested for preference of differing saline concentrations, stress induced analgesia and open-field activity. We found that IMP-ADX animals did not require supplemental NaCl for survival, unlike ADX rats. However, both IMP-ADX and ADX groups exhibited a preference for saline greater than controls at all concentrations (0.5, 1.0, 1.5 and 3.0% NaCl). Stress induced analgesia was produced by immobilization for one hour prior to testing for latency to move the tail away form a radient heat source. Results show a reduced response latency for both IMP-ADX and ADX groups. Activity in an open-field was monitored during a five minute testing period. Animals in the ADX and IMP-ADX groups showed a tendency to be hypoactive compared to controls.

Anatomical examination of adrenal implants showed good survival of the tissue, with a 5-10 fold increase in size following removal of adrenal glands. The adrenal implants appeared to maintain their normal structure, consisting of cortex and medulla. Also, adrenal implants showed met-enkephalin immunoreactivity. AChE positive staining indicates implants receive cholinergic innervation from the host brain.

Transplanted adrenals appeared to maintain their normal structure and increased in size in response to peripheral adrenal loss. Behavioral results may be interpreted as indicating that central adrenal transplants do not function to restore behavioral deficits induced by adrenalectomy, although they can assume some function in regulation of body fluids.

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288.12 CROSS-SPECIES SEPTAL TRANSPLANTS: THE RELATIONSHIP BETWEEN THE RESTORATION OF ChAT AND AChE ACTIVITY IN HOST HIPPOCAMPAL FORMATION. J.K. Daniloff, R.P. Bodony*, W.C. Low, J. Ellis and J. Wells. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

In our studies of xenogenic transplantation of neural tissue between two species of rodents, we have described the time course for the reestablishment of the host's acetylcholinesterase (AChE) laminar pattern in the hippocampus (Anat. Rec. 14: 49A, 1983); and shown the restoration of a complex behavior altered through hippocampal cholinergic denervation (Soc. Neurosc. Abst. 9: 859, 1983). In this report we determine the transplant-induced return of choline acetyltransferase (ChAT) activity within the host hippocampus, and describe the temporal relationship between ChAT and AChE.

For the ChAT analysis, cell suspensions were created from nerve cells dissected from the developing septal region of mouse embryos (E15-17) and transplanted into 18 rats. A bilateral fornix-fimbria transection was performed in each rat and one hippocampus received a unilateral, single 5 µl injection of the cell suspension. The side opposite the transplant served as a lesioned control. Following survival periods of 1, 4, and 17 weeks, each hippocampus was dissected free and divided into three approximately equal pieces (rostral, middle, and caudal segments). Segments were assayed separately using the rapid radiochemical method of Fonnum (J. Neurochem. 24: 407, 1975). Six hippocampi from unoperated animals provided a measurement of normal activity.

ChAT activity recovered gradually as survival time increased. Highest activity was 35% of normal after 17 weeks and was localized within the caudal segments. The caudal segment is the one closest to the transplant. In rostral and middle segments activity was significantly greater than lesioned controls, but only after 17 weeks. In caudal segments activity was significantly greater after both 4 and 17 weeks. A single transplant restored 23% of normal activity after 17 weeks when averaged over the whole hippocampus.

In contrast, the density of AChE-ingrowth decreased from the 1st to 3rd weeks of survival, but increased between the 3rd and 4th weeks. Density of AChE stabilized from the 4th to 17th weeks. The greatest changes in ChAT occurred after the density of AChE had stabilized. The time course for the recovery of maze behavior more closely paralled the recovery of ChAT activity than it did AChE. Supported by PHS #5429-17-3 and a grant from the American Federation for Aging Research.

288.13 THE EXPRESSION OF GFA PROTEIN SYNTHESIS IN DONOR AND HOST ASTROCYTES FOLLOWING TRANSPLANTATION. L.M. Smith and F.F. Ebner, Center for Neural Sciences and Div. of Biol. & Med. Brown University, Providence, R.I. 02912

The response of immature and mature astrocytes to transplantation may be an important factor in controlling the growth of axons into and out of the donor tissue. We have studied the response of astrocytes in embryonic donor neocortex and in adult host neocortex at various times after implantation (BALB/c mice). Astrocytes were characterized immunocytochemically by their production of glial fibrillary acid protein (GFAP) (AB provided by Dr. L. Eng). Normally, GFAP immunoreactivity is expressed in high concentrations by cortical radial glial cells and astrocytes only from E18 to PND10-14 in mice (Woodhams, et al., Anat. & Embry., 163:331, 1981). Normal adult neocortex shows detectable GFAP-positive cells mainly in layer I and white matter. Damage to adult cortex alone, such as that required for the transplantation procedure, produces active GFAP production which spreads to astrocytes located throughout the hemisphere, but by 3 weeks the reactive astrocytes are restricted to the vicinity of the lesion (Berry, et al., Acta Neurochir. Suppl. 32:31, 1983). When transplants are inserted in the lesioned area, the host brain GFAP response is nearly completely suppressed. Only a small number of immunoreactive astrocytes are seen in the host brain around healthy transplants after 30 days or longer survival periods. In contrast, astrocytes remain intensely GFAP-positive within the transplants for over 6 months. Transplants from younger donors aged E12-14 develop a higher density of reactive astrocytes than older E17-19 donor tissue. We conclude, first, that some feature of the transplanted embryonic tissue actively suppresses the GFAP response that usually follows damage to adult cortex, and second, that some mechanism which normally sharply decreases the production of GFAP at 1-2 weeks after birth is missing or at least ineffective in turning off GFAP production in the developing transplants (Supported by NIH grant #NS13031).

MALNUTRITION AND BRAIN DEVELOPMENT

289.1 EFFECTS OF PRENATAL AND EARLY POSTNATAL MALNUTRITION AND LATER CORTICAL LESIONS ON LEARNING BEHAVIOR OF RATS. S. Finger, C.R. Almli, A. Bouzrara*, M. Silva*, and D. Waksman*. Dept. Psychology, Washington University, St. Louis, MO. 63132

Early dietary history may be one of the factors contributing to the variability in performance typically seen after "comparable" lesions in clinical populations. As a test of this idea, we now have studied the effects of frontal cortical lesions sustained by rats that had early dietary histories of severe or moderate malnutrition, or normal nutrition.

Dams were prenatally and postnatally malnourished (severe= 6% casein diets, moderate= 8% casein diets), with dietary rehabilitation (25% casein diets) for the pups begun at 21-40 days of age. These malnourished and normally nourished (25% casein diets throughout life) rats received frontal cortical lesions or control operations at 90 days of age. The animals were then tested for acquisition and 3 reversals of a tactile discrimination (rough-smooth).

Main effects of lesion and nutrition were found for both the acquisition and reversal learning measures on the tactile discrimination. The groups with malnutrition plus frontal cortical lesions displayed the poorest performance, indicating additive effects of lesion and nutrition. The severely and moderately malnourished groups did not differ from each other for the acquisition of the discrimination, but the severely malnourished rats made more reversal errors than the moderately malnourished group. The severely malnourished group showed greater brain growth retardation (size and weight) than the moderately malnourished group.

This research supports the hypothesis that early dietary history can affect the response to a brain lesion sustained later in life. These results are consistent with research showing that animals that are born with low-birth-weights often display more severe reactions to brain injuries than do rats born at normal-birth-weights. Thus, the variability in sparing and/or recovery of function following comparable lesions in clinical populations may be at least partially related to alterations in neural plasticity as a function of early dietary history. (Supported by BRSG-7054 and HD-06364 Grants)

289.2 PERFORMANCE DEFICITS ON BEHAVIORAL TASKS INVOLVING SPATIAL CUE UTILIZATION FOLLOWING CHRONIC PROTEIN RESTRICTION OF RATS. C.R. Goodlett, M.L. Valentino*, O. Resnick and P.J. Morgane. Worcester Found. for Expt. Biol., Shrewsbury, MA 01545

Spatial cue utilization of developmentally malnourished and well-nourished rats was examined in a series of tasks known to be sensitive to damage to the hippocampus and other limbic structures. Malnourished rats were born to dams fed an 8% casein diet beginning 5 weeks before mating, while the well-nourished rats were born to dams fed an isocaloric 25% casein diet. The respective diets were maintained during lactation and after weaning. Malnourished rats tested for spontaneous alternation in a T-maze showed no evidence of alternation deficits relative to well-nourished rats. However, in the experiments involving rewarded alternation the 8% rats committed significantly more repeated errors than the 25% rats. Spatial localization capabilities were explicitly tested in a series of four experiments using the spatial navigation problem devised by Morris (1981). In the absence of any pretraining or experience with swimming the 8% males and females took significantly more trials to reach a performance criterion (Exp. 1). Extended experience with swimming in a different apparatus attenuated but did not eliminate the acquisition deficits in the Morris maze (Exp. 2). However, following the initial training with the platform in one location in this experiment, the 8% and 25% groups showed equal rates of learning when the position of the platform was changed from day to day. Rats given the 8% diet beginning in adulthood showed no evidence of the previously demonstrated acquisition deficits, indicating the importance of the developmental timing of the nutritional manipulation (Exp. 3). Using 4 rather than 8 trials per day in experienced swimmers eliminated acquisition differences in the developmentally malnourished rats (Exp. 4). Additionally, both groups showed evidence of latent learning when given extensive exposure to the location of the platform prior to initial acquisition training (Exp. 4). Thus, while the spatial localization abilities of malnourished rats are intact, developmentally malnourished rats apparently have difficulty in selecting the appropriate spatial response strategies when the task demands are relatively difficult or unfamiliar. While these behavioral tests do not indicate a global hippocampal dysfunction resulting from early malnutrition, they do implicate an impairment of stimulus selection or attentional processes. (Supported by NIH Grant HD-06364).

289.3 PRENATAL PROTEIN MALNUTRITION: EFFECTS ON HIPPOCAMPAL KINDLING.

R. J. AUSTIN-LAFRANCE *, JOSEPH D. BRONZINO, CHRISTOPHER MELO *, PETER J. MORGANE. (SPON. E. SHASKAN). TRINITY COLLEGE, HARTFORD CT. 06106 and THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY, SHREWSBURY, MA. 01545.

Recent studies have shown that animals subjected to malnutrition are more susceptible to seizures than animals raised on a normal diet (Taber et al, Experientia, 36: 69-70, 1982). Kindling refers to the increasing duration and spread of a characteristic afterdischarge pattern in the EEG in response to repeated electrical stimulation of the brain. The kindling process elicits behaviorally identifiable stages through which the animal progresses, generally culminating in a full motor seizure. Given these factors, the present study was undertaken to evaluate the effect of prenatal protein malnutrition on the development of hippocampal kindling in the rat. Specifically, we have quantified the gradual development of electrically induced seizures in rats born to dams maintained on either a 25% or 6% casein protein diet and kept on this diet throughout life. In this study, animals raised on the 6% casein protein diet, in comparison to the 25% diet group had: (1) a significantly lower ($p < .01$) threshold stimulus intensity to afterdischarge and; (2) a significantly higher ($p < .01$) mean duration of afterdischarge at all phases of the kindling process. Animals reared on the protein deficient diet reached specific behavioral stages earlier than the 25% diet group with the exception of the final, motor seizure stage. In this latter case, animals reared on the 25% casein protein diet required an average of 22 stimulations to reach motor seizure while several animals in the 6% diet group failed to reach the motor seizure stage even after as many as 40 stimulations. The inability to reliably kindle animals in the 6% protein group parallels similar findings in immature animals, (Gilbert and Cain, Dev. Brain Res., 2:321-328, 1982) suggesting that protein malnutrition may retard the maturation of neuronal mecanisms involved in the kindling response.

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289.4 LONG-TERM POTENTIATION OF THE EXTRACELLULAR POST-SYNAPTIC POTENTIAL IN THE DENTATE GYRUS OF THE PROTEIN MALNOURISHED RAT. K.Austin*, O.Resnick and P.J. Morgane, Worcester Found. for Expt. Biol., Shrewsbury, MA 01545.

In our long-term studies of effects of protein malnutrition on the central nervous system we have investigated the phenomenon of long-term hippocampal potentiation(LTP). Field potentials evoked by stimulation of the perforant path were observed in the molecular layer of the dentate gyrus of chronically implanted adult rats born to dams fed either 6% or 25% casein diet initiated 5 weeks prior to mating and continued through pregnancy and lactation. The pups were maintained on these diets after weaning and into adulthood. Under Nembutal anesthesia, electrodes were placed in the frontal cortex, entorhinal cortex, and dentate gyrus. Placement of the electrode in the dentate gyrus was aided by monitoring multiple unit discharges. Placement in the perforant path was accomplished by adjusting the stimulating electrode until the field potential was maximum. One week later the animals were given test stimulations to determine baseline field potentials. In the first experiment current levels were adjusted to give an EPSP wave with a peak between 1 and 1.5 mv (width of 20 μsec) for each animal. After baseline measures were obtained conditioning stimulation was given for 0.5 second at 100 Hz. The next day, baseline measures were again taken to determine degree of potentiation. In this experiment, the control animals showed potentiation of the EPSP slope of 228% while no potentiation was observed in malnourished animals. A second experiment used pulsed conditioning stimulation to initiate LTP. Here, the control animals exhibited a potentiation of the EPSP slope of 61% whereas malnourished animals potentiated only 15%. Baseline measures revealed that stimulation needed to meet the EPSP wave height criteria (1-1.5 mv) was as much as 3 times greater in malnourished as in control animals. The field potential represents a population response and, therefore, one possible explanation is a decreased number of cells responding in the area of the recording electrode. This view is supported by work showing a 17% reduction of cells in the dentate gyrus of malnourished rats (Jordan & Clark, 1982). These results suggest that malnutrition may cause a deficit in establishing LTP. Additional support for this view is shown by Jordan & Clark (1983) who demonstrated a deficit in establishment and maintenance of LTP of the population spike. Studies are underway to determine how LTP develops in other hippocampal fields and when conditioning stimulation is administered in vigilance states other than waking (supported by NIH grant HD-06364).

289.5 THE EFFECT OF PROTEIN MALNUTRITION OF THE VIGILANCE STATE DEPENDENT MULTI-UNIT ACTIVITY RECORDED FROM THE HIPPOCAMPAL FORMATION OF THE RAT Joseph D. Bronzino, C.J.Siok*, K. Austin* and P.J. Morgane Trinity College, Hartford, CT, 06106, and the Worcester Foundation for Experimental Biology, Shrewsbury, MA, 01545.

Previous studies have indicated that prenatal protein malnutrition significantly effects the development of the hippocampal EEG in the rat (Bronzino et al, EEG Clin. Neurophys., 55:699-709, 1983). To further study these effects, we have examined the vigilance state dependent multi-unit activity (MUA) recorded from the pyramidal cells of the dorsal hippocampal field CA1 in rat pups born to dams fed either a normal (25% casein) diet, or a protein deficient diet (6% casein) throughout a 5-week pregravid period, gestation and lactation. After weaning, offspring were maintained on the same diet as the mother. At 100-120 days of age, the animals were chronically implanted with fixed, microwire multi-unit recording electrodes positioned in the pyramidal cell layer of CA1. An EEG recording electrode was also implanted in the region of the frontal cortex. Multi-unit activity was assessed by counting the frequency of discharges which occurred above a single threshold set to yield an average firing rate of 20 spikes/sec during the vigilance state of quiet waking (QW). Using this threshold, MUA was analyzed for the vigilance states of slow-wave sleep (SWS) and REM sleep, and all results were reported as the percent change in firing rate compared to QW. We found that in the animals reared on the 25% casein diet, there is a decrease in the firing rate of these neurons during REM, and a significant ($p < .01$) increase (22%) in the firing rate during SWS. In the malnourished animal, there was a similar decrease in MUA recorded during REM, but a much higher increase (130%) in the firing rate of the CA1 pyramidal cells during SWS. These results indicate that protein malnutrition significantly alters the cellular activity of the pyramidal cells of CA1 during SWS, which may be a consequence of changes in the modulation of hippocampal neuronal activity by brainstem structures.

Supported by NIH Grant HD 06364

289.6 ALTERED DEVELOPMENT OF RESPONSIVENESS TO CLONIDINE IN PRENATALLY MALNOURISHED RATS. M.L. Valentino*, C.R. Goodlett, P.J. Morgane and O. Resnick. (Spon: W. McFarland). Worcester Found. for Expt. Biol., Shrewsbury, MA 01545.

Our group has shown that protein restriction to rats during gestation results in marked elevations of brain norepinephrine and serotonin at birth which are not reversed by cross-fostering to well-nourished lactating dams. The functional impact of these early, long-lasting elevations were examined using the alpha-2 agonist clonidine as a probe of the ontogeny of alpha-2 noradrenergic mechanisms. Developing rats typically exhibit a dramatic change in response to clonidine, from motor activation and wall-climbing elicited during the first two weeks of life to a cataleptic-like suppression of activity by the end of the third week. Thus, we examined the behavioral effects of clonidine administered at postnatal days 5, 10, 15 and 20 to rat pups born to and reared by dams maintained on either a 25% casein diet or an isocaloric 6% casein diet. Separate litters were tested at each age and each pup was given either the saline vehicle or a single dose of clonidine (0.1, 0.5, 1.0 or 2.5 mg/kg). A "blind" rater scored the behavioral activity for 90 minutes using a 30 second time-sampling procedure. Both the 6% pups and the 25% pups given clonidine showed a dose-dependent behavioral activation at 5 and 10 days, in terms of total activity counts and in specific wall-climbing counts. However, the 6% pups were less responsive to clonidine at these ages as indicated by a shift to the right in the dose-response curve, suggesting a subsensitivity to clonidine at these ages. At 15 days both nutritional groups were activated, but to a lesser and more variable extent than the previous ages. Clearcut differences between the 6% and 25% groups were found at 20 days. The pups of the 25% diet showed the expected suppression of behavioral activity to all doses of clonidine while the 6% pups continued to show behavioral activation and wall-climbing at this age. The results indicate a delay in the malnourished pups in the functional maturation of the substrate mediating the effects of clonidine stimulation, likely reflecting a delay in the functional ontogeny of systems regulated by alpha-2 receptors. We are presently examining the effects of clonidine at later ages in rats on the 6% and 25% casein diets, and in prenatally malnourished groups cross-fostered to well-nourished dams at birth. (Supported by NIH Grant HD-06364).

289.7 ELEMENTAL COMPOSITION OF BRAIN AND OTHER ORGANS FROM RATS FED A MARGINAL ZINC-DEFICIENT DIET IN UTERO AND DURING LACTATION. J.C. Wallwork*, E.S. Halas and D.B. Milne* (SPON: S.S. Parmar). USDA, ARS, Human Nutrition Research Center and Dept. of Psychology, Univ. of North Dakota, Grand Forks, ND 58202

Marginal levels of dietary zinc fed during gestation and lactation caused permanent memory impairment with accompanying abnormalities in hippocampal development in rats. Also, severe zinc deficiency causes elemental changes (other than zinc) in the brain of growing rats. Consequently, we examined the elemental composition of the brains, livers and femurs of rats fed 10 ppm dietary zinc in utero and during lactation. The biotin-enriched diet contained 20 % egg white and was supplemented with inositol (1 mg/kg). Female rats (240 g) were fed a stock laboratory diet and were mated. From the day of conception, the rats were fed the diet containing 10 ppm zinc and distilled deionized water (ZD group). This diet was fed throughout gestation and lactation. Pair-fed (PF) and ad libitum-fed (AL) zinc-supplemented control rats were fed the same diet and water containing 25 ppm zinc. On the day after parturition (day 1) the pups were culled to nine per litter and organs taken for elemental analysis. After 23 days the pups were weaned. At this time the dam and one male rat from the litter were killed and organs taken for elemental analysis. The remaining animals were rehabilitated by feeding stock laboratory diet for 100 days when one male rat from each litter was killed and the brain taken for analysis. The nutritionally rehabilitated offspring were trained on a 17-arm radial maze starting at 100 days. The ZD rats were significantly impaired in their working memory and learning when compared to PF and AL rats. The PF rats were not impaired. Perinatal zinc deficiency led to depressed levels of zinc in livers and femurs of 1-day-old and 23-day-old offspring. Brain zinc concentrations conversely, were not depressed by this treatment in these animals or in adult animals which had been rehabilitated by feeding a stock laboratory diet. Likewise the other brain elements examined did not show any consistent changes at 1, 23 or 100 days of age in the zinc-deficient rats. Apparently, changes in elemental composition do not occur at this mild level of deficiency and do not appear to be related to the permanent memory deficits produced by perinatal zinc deficiency.

289.8 BRAIN GROWTH IN RAT PUPS REARED UNDER DIFFERENT FEEDING CONDITIONS E. Moore*, E. Murowchick* and J. Diaz. Dept. of Psychology, University of Washington, Seattle, WA 98195.

Nutritional insults have been shown to severely retard somatic growth in rats, with less severe effects on brain growth. Dobbing and Sands (Biol. Neonate, 1971) have hypothesized that the brain is most susceptible to the effects of undernutrition during its period of fastest growth. Animals reared in isolation and fed exclusively via chronic intragastric cannulas during this period of accelerated brain growth have been shown to have a 8-12% brain weight deficit compared to their normally reared siblings despite comparable body weights (Diaz et al., Br Res Bull, 1983). The purpose of this study was to investigate the brain effects of nutritional insults in normally reared (NR) vs. gastrostomized (AR) rat pups at different stages of brain development.

A 2^4 factorial design was used. The four factors included 1) rearing condition (AR vs. NR), 2) gender, 3) nutritional status (undernourished vs. normally nourished and 4) day of sacrifice (day 10 vs. day 18). Four day old Long-Evans rats were assigned at random within each gender to 1 of the 8 treatments. The technique for gastrostomy rearing has been described previously (Diaz et al., J. Nutr., 1982). Undernutrition in the NR groups was produced by placing the pups with a nonlactating female for 8-10 hours per day. All animals were examined daily for eye opening and incisor eruption. At the time of sacrifice, each animal's brain, liver, kidney and spleen were removed and weighed. All organs were then wrapped in aluminum foil and placed in a 85°C oven to dry.

There were no significant body weight differences on day 4. The undernourished animals were 20% smaller than the normally nourished animals when sacrificed ($p<.01$). There was no difference in body weight due to rearing condition. The brains of the AR pups were 14% smaller than the brains of the NR pups ($p<.01$). There was no brain weight difference due to level of nutrition. These data indicate that when nutritionally challenged, artificially reared animals do engage a brain sparing mechanism as do normally reared animals. Furthermore, since this mechanism did not compensate for the overall reduction in brain weight imposed by the artificial rearing procedure, the data also suggest that the brain weight deficits typically observed in AR animals are not caused by mere nutritional restriction. (Supported by NSF grant PRM-8114914)

289.9 ACUTE NUTRITIONAL DEFICITS IN EARLY BRAIN DEVELOPMENT. C. Stamper, E. Moore* and J. Diaz. Dept. of Psychology, University of Washington, Seattle, WA 98195.

When availability of nutrients is restricted early in life, brain growth is less severely affected than growth of other organs. The purpose of this study was to describe the effects of varying concentrations of a replacement diet on early brain growth. This study was conducted over a 24 hour period in rat pups that were fed exclusively by chronic intragastric cannulas.

Four day old Female Long-Evans rat pups were assigned by weight to one of the following groups: 1) animals artificially reared with a milk-formula (the procedural and formula details have been previously described (Diaz, et al., J. Nutr., 1982)) (AR-PM, n=14); 2) animals artificially reared with this formula diluted by 59% (AR-DIL, n=12); 3) animals artificially reared receiving only water and no formula (AR-WATER, n=10); or 4) animals normally reared (NR, n=12). The animals in group 2 received 59% more formula than did the animals in group 1 so that both these groups received the same absolute amount of the formula. The animals were sacrificed 24 hours after cannula implantation. Each animal's brain, liver, kidney, and spleen were removed and weighed. The following table summarizes the results:

	Mean Weight (grams)				Ratio
group	body	brain	cerebellum	liver	brain:body
1. AR	12.1	.47*	.02	.42	.041
2. AR-DIL	12.4	.49	.02	.41	.042
3. AR-WATER	10.6**	.50	.02	.28**	.048
4. NR	12.4	.49	.02	.40	.042

(* $p<.05$; ** $p<.01$; whole brain minus cerebellum)

These data suggest that the acute brain weight deficits previously observed in the artificially reared animals are due to the high osmolarity of the replacement formula. The brain growth of the AR-WATER animals is in sharp contrast with the brain growth of the AR animals. The AR-WATER animals had a vigorous brain sparing effect whereas the AR animals showed decreased brain growth along with normal body growth indicating a brain sparing response was not engaged. The acute effects observed in this experiment seem to be different from the long term effects seen after two weeks of artificial rearing. Thus the specific mechanisms underlying brain growth at various ages early in development appear to be dynamic and diverse.

(Supported by NSF grant PRM-8114914)

289.10 ABNORMAL AUDITORY EVOKED POTENTIALS IN MALNOURISHED INFANTS. A. Barnet, I. Weiss, Children's Hospital National Medical Center, Washington, D.C. 20016, J. Flinn*, K. Perkins*, and Z. Tyer*, George Mason University, Fairfax, VA 22030.

Cortical auditory evoked potentials (AEPs) were recorded bilaterally at C_3 and C_4 from severely malnourished infants at admission to hospital and at discharge, together with AEPs from infants in the hospital day care center.

At admission, the malnourished infants had significantly fewer peaks in the AEPs to clicks recorded at C_3, over the left hemisphere, than age-matched controls. ($t \geq 3.8$, $df = 43$, $p < .001$). There was no significant difference for AEPs recorded at C_4, over the right hemisphere. At discharge the malnourished infants had significantly more peaks than at admission for the AEPs recorded both a C_3 ($t = 2.15$, $df = 43$, $p < .05$) and at C_4 ($t = 2.0$, $df = 43$ $p < .05$). There were no significant differences between the number of peaks for age-matched controls and the previously malnourished infants after discharge from hospital at either C_3 or C_4.

The data show that malnutrition in infancy affects cortical AEPs and that recovery of AEPs parallels improved nutritional status.

289.11 EFFECT OF UNDERNUTRITION ON NON-PYRAMIDAL CELLS OF VISUAL CORTEX IN RATS OF THREE AGE GROUPS. S. Díaz-Cintra, L. Cintra, T. Kemper, and P.J. Morgane. Worcester Foundation for Experimental Biology, Shrewsbury MA, 01545 and Instituto de Investigaciones Biomédicas, UNAM, Dept. de Fisiología, 04510, México, D.F.

Using Rapid Golgi technique and morphometric analysis in three cell types: sparley spinous multipolar, bitufted and bipolar non-pyramidal cells, in layer IV of the visual cortex, we studied their major and minor axis of the cell body, the number, diameter linear extent and spine density in a 50 microns segment in primary, secondary and terminal dendrites; in 25% and 6% casein diet rats at 30, 90 and 220 days. The most striking findings was found in synaptic spine density on three dendritic segments. The sparsley spinous multipolar cell in controls, showed a marked age-related decrease in all three dendritic spines between 30 and 90 days and a marked increase between 90 and 220 days. In contrast, protein deprived rats, presented very little age-related change, seemingly, participating little in these dramatic shifts. The bitufted sparsley spinous cells in controls, shoed a steady increase in primary and secondary synaptic spines from 30 to 220 days, while the 6% rats presented an increase from 30 to 90 days and then a decrease. On terminal dendrites control showed a slight decrese from 30 to 90 days, then a marked increase, while the undernourished rats presented a progressive decrease. The bipolar sparsley spinous cells in control rats showed no consistent pattern on these three dendritic segments. Protein deprived rats showed a decrease in synaptic spine density from 30 to 90 days in all three dendritic segments with a further decline in the density on primary dendrites and an increase on secondary and terminal dendrites from 90 to 220 days. Age-related changes in linear extent showed most marked effect on bipolar sparsley spinous 6% cells and presented a consistent deficit at all ages for all three dendritic segments. Another striking finding in 6% casein rats as compared to controls was a marked increase in neuronal cell size on the bitufted cell at 90 days and on the bipolar cells, at 30 days. With few exceptions most cells showed a decrease in dendritic diameter and a variable pattern in number of dendritic processes. These data shows a clear individuality of these three cell types and how each cell reacts different to 6% casein diet. (Supported by NIH Grant HD-06364).

289.12 REGIONALLY SELECTIVE ALTERATIONS OF IN VITRO CARBOHYDRATE METABOLISM DURING THIAMIN DEFICIENCY. G. Gibson, P. Nielsen*, V. Mykytyn*, and J. Blass. Department of Neurology, Cornell University Medical College, Burke Rehabilitation Center, White Plains, NY 10605.

Thiamin deficiency in man causes confusion and memory deficits by unknown neurochemical mechanisms. Only certain brain regions show pathological changes. Similar selective histological changes occur in animal models of thiamin deficiency. To evaluate the molecular basis of this regional vulnerability to thiamin deficiency, the in vitro carbohydrate metabolism of tissue from damaged (mammillary bodies and inferior colliculi) and non-damaged (cochlear nuclei) brain regions was compared. Thiamin deficiency was induced in rats by a combination of dietary thiamin deprivation and pyrithiamin injections. Animals were sacrificed after 11 days (minimal weight loss and pathological changes) or 14 days (severe histological, weight and behavioral changes). Metabolism was assessed by $^{14}CO_2$ production from [3,4-^{14}C]-glucose (an index of oxidation by pyruvate dehydrogenase), [2-^{14}C]glucose (a measure of flux through the citric acid cycle) and [U-^{14}C]glucose (an indicator of overall glucose metabolism). [U-^{14}C]Glucose incorporation into an acid insoluble fraction was also determined. On day 11, mammillary body metabolism was unaltered except for [2-^{14}C]glucose (82±6% of control), whereas inferior colliculus showed decreased (% of control ± S.E.M.) $^{14}CO_2$ production from [U-^{14}C]glucose (82±4%), [3,4-^{14}C]glucose (78±3%) or [2-^{14}C]glucose (83±3%) and diminished (84±3%) [U-^{14}C]glucose incorporation into the acid insoluble precipitate. After 14 days of treatment, the metabolic alterations in the mammillary bodies and inferior colliculi were severe and they were more than in the cochlear nucleus. Decreases (% of control ± S.E.M.) occurred in $^{14}CO_2$ production from [U-^{14}C]glucose (30±2, 33±2 and 68±8 in the mamillary bodies, inferior colliculi and cochlear nuclei, respectively), from [3,4-^{14}C]-glucose (40±6, 48±5 and 77±5) or from [2-^{14}C]glucose (54±14, 49±5 and 78±6). Only mammillary bodies showed diminished [U-^{14}C]glucose incorporation into acid precipitable material (59±7). Thus, thiamin deficiency alters in vitro carbohydrate metabolism the most severely in brain regions that will develop pathological changes.

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OCULOMOTOR SYSTEM III

290.1 SELECTIVE RETROGRADE TRANSNEURONAL TRANSPORT OF LECTIN/HRP CONJUGATES IN THE OCULOMOTOR SYSTEM. J.D. Porter, B.L. Guthrie, and D.L. Sparks. Dept. Anat., Univ. of Mississippi Med. Ctr., Jackson, MS 39216 and Dept. Physiol./Biophys., Univ. of Alabama in Birmingham, Birmingham, AL 35294.

Recent studies have identified second-order sensory neuronal somata by anterograde transneuronal transport of wheat germ agglutinin-conjugated HRP (WGA/HRP), but have failed to demonstrate retrograde transneuronal transport of WGA/HRP. Since morpho-physiological studies in the cat have characterized premotor neurons that terminate on abducens motoneurons, the oculomotor system is an appropriate model in which to examine the possibility of retrograde transneuronal transport of WGA/HRP.

Injections of WGA/HRP into the monkey (Macaca mulatta, M. arctoides) lateral rectus muscle produced a pattern of retrogradely labelled motoneurons like that noted in our earlier studies (J. Comp. Neurol. 198,'81; 218,'83). In contrast to data obtained with native HRP, WGA/HRP injections also resulted in labelled neurons in the ipsilateral medial vestibular nucleus and the contralateral reticular formation. These regions correspond to sites of inhibitory vestibular neurons (IVNs) and inhibitory burst neurons (IBNs), both of which are known to terminate on abducens motoneurons. These studies failed to label any other population of premotor neuron. Labelled neurons were absent from sites of either excitatory burst (EBNs; ipsilateral reticular formation) or excitatory vestibular (EVNs; contralateral medial vestibular nucleus) neurons. In addition, labelled neurons were not observed in nucleus prepositus hypoglossi and the oculomotor complex.

These data are consistent with the notion of retrograde transneuronal transport of WGA/HRP to premotor neurons following its peripheral uptake by abducens motoneurons. A specific transneuronal transport mechanism is suggested by the finding that only certain populations of premotor neurons were labelled. Individual neurons within the transneuronally labelled populations (IVNs and IBNs) are known to contact many motoneurons and such contacts are proximal on the motoneuron soma/dendritic tree. By contrast, individual EVNs and EBNs contact fewer motoneurons and/or their synaptic terminals are situated on more distal dendrites. The observed pattern of transneuronal transport therefore may be dependent upon the synaptology of premotor neurons.

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290.2 THE OCULOMOTOR SYSTEM OF THE GOLDFISH, CARASSIUS AURATUS. James F. McGurk and Werner Graf. The Rockefeller University, New York, N.Y. 10021.

Peripheral and central oculomotor organization of the goldfish was studied. The area and number of fibres in cross-sections of the extraocular muscles were quantified and their kinematics determined. Locations of extraocular motoneurons were found by retrograde transport of horseradish peroxidase (HRP) injected into the extraocular muscles. Individual morphology of motoneurons was visualized by intrasomatic injection of HRP. The macrospic appearance and kinematics of the muscles had the characteristics of lateral-eyed animals (e.g. rabbit). All the muscles were of similar size. The eye muscles were innervated by four ipsilateral (lateral rectus, medial rectus, inferior oblique, inferior rectus) and two contralateral (superior rectus, superior oblique) motoneuron pools. The oculomotor nucleus was found in the midbrain, at the level of the caudal zone of the periventricular hypothalamus. Inferior rectus motoneurons were placed most rostrally in the oculomotor complex, whereas medial rectus, superior rectus and inferior oblique motoneurons were intermingled in the more caudally located portions. All labelled cells were located dorsally and medially to the medial longitudinal fasciculus (MLF) in close proximity to either the floor of the ventricle or the midline region. Occasionally, motoneurons were interspersed within the fiber bundles of the MLF or the exiting fibers of the oculomotor nerve. The trochlear nucleus, containing superior oblique motoneurons was found in the immediate lateral and caudal neighborhood of the oculomotor nucleus, its rostral border overlapping with the caudal border of the latter. Axons of superior rectus motoneurons crossed the midline without any detour to enter the contralateral oculomotor nerve. In contrast, trochlear motoneuron axons arched around the dorsal aspect of the ventricle through the cerebellum to reach the contralateral trochlear nerve. The abducens nucleus containing lateral rectus motoneurons was located in the posterior brain stem in the neighborhood of the vestibular nuclear complex. This nucleus was divided into a rostral and a caudal subdivision. The large dentrites of motoneurons were laterally polarized. The axons did not collateralize within the midbrain region or the oculomotor nerve as far as they could be traced. The oculomotor system of the goldfish is similar to the situation found in other teleosts and higher vertebrates, displaying characteristics of an animal living in a three-dimensional environment. Supported by NIH grant EY04613.

290.3 A "TRIGEMINAL" INPUT TO THE SUPRAOCULOMOTOR AREA. P. J. May, P. P. Vidal*, H. Baker and R. Baker. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016; Lab. Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021.

The supraoculomotor area (SOA), a region of the periaqueductal grey located immediately above the oculomotor nuclei, is thought to play a role in oculomotor function. In fact, the SOA contains dendritic arbors extending from motoneurons within the oculomotor nucleus and axon collaterals from tectal and vestibular neurons. Moreover, following injections of HRP centered in the abducens nucleus, retrogradely labelled neurons are concentrated just above the oculomotor nuclei in the caudal portion of the SOA. In our present experiments in cats, an afferent input to the SOA from the lateral tegmentum was found following injection of HRP centered in the pontine sensory trigeminal complex. Axons of labelled fibers crossed the midline and traveled rostrally and then dorsally to terminate in the periaqueductal grey capping the contralateral, caudal oculomotor nucleus. To ascertain the exact source of this pontine projection, small iontophoretic injections of HRP were placed in the caudal SOA. A column of small and large retrogradely labelled neurons was found adjacent to the pontine sensory trigeminal nuclei, and their location can be best described as investing the exiting Vth and VIIth nerves. These neurons are equivalent to those described by Graybiel and Hartwieg (1974) as lying in the pontine lateral tegmentum and extending into the Kölliker--Fuse nucleus, a region which contains catecholaminergic neurons. To determine whether the pontine projection to the SOA was catecholaminergic, sections were incubated in TMB and then in antibody to tyrosine hydroxylase, with DAB as the chromagen. Although catecholaminergic and retrogradely labelled cells were intermingled in the lateral tegmentum, no double labelled cells were found. Therefore, we conclude that the cells projecting to the supraoculomotor region are not catecholaminergic and are, on the basis of their location, more likely to be part of the trigeminal complex. If so, this pontine input to a specific supraoculomotor area that contains cells projecting to the abducens nucleus may be part of a pathway related to eye movements occurring during blinking. Supported by NIH grants EY05689, EY02007 and NS13742.

290.4 ARE SUPRAOCULOMOTOR NEURONS PROJECTING TO THE ABDUCENS NUCLEUS SEROTONERGIC? P.P. Vidal*, P.J. May, H. Baker and R. Spencer. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016; Lab. Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021; Dept. Anatomy, Med. Coll. of Virginia, Richmond, VA 23298.

The supraoculomotor area (SOA) of the periaqueductal grey lies above the oculomotor and trochlear nuclei, and contains neurons projecting to the abducens and accessory abducens nuclei. Serotonergic cells have also been found in the SOA, and serotonergic terminals are present in both target nuclei. We determined the extent to which these two populations overlapped using a double label technique. TMB was employed to reveal cells labelled retrogradely from HRP injections in the abducens nucleus and serotonergic neurons were localized by employing a serotonin antibody with DAB as chromagen. Essentially, there were no double labelled cells present and, more significantly, the two populations had different, though adjacent, distributions in the SOA. Retrogradely labelled cells were concentrated in an area which capped the caudal oculomotor nucleus, and they were also scattered within the nucleus proper. The serotonergic cells were distributed more caudally in the region between the trochlear and oculomotor nuclei. They were concentrated in raphe dorsalis, a fountain-shaped region which runs from the surface of the aqueduct down the midline between the trochlear nuclei. In complementary electrophysiologic experiments, intracellular staining with HRP revealed a set of neurons whose distribution and morphology were very similar to the serotonergic population. Both had small elongated somata oriented parallel to the ventricular surface with several long, sparsely branched dendrites extending across the periaqueductal grey. These putative serotonergic neurons could not be activated either anti- or orthodromically from electrical stimulation in the abducens nucleus, nor did they receive vestibular input. Conversely, cells in the SOA antidromically activated from the abducens nucleus displayed a distinctly different morphology when intracellularly stained and reconstructed. Taken together this evidence strongly suggests that the caudal SOA contains serotonergic neurons which are separate from the population projecting to the abducens nucleus and we conclude they are probably not directly involved in the production of eye movements. Supported by NIH grants EY05689, NS13742 and EY02191.

290.5 BRAINSTEM AFFERENTS TO THE ABDUCENS NUCLEUS IN THE MONKEY. T. P. Langer* and C. R. S. Kaneko. Dept. of Physiology & Biophysics and Regional Primate Research Center, Univ. of Washington, Seattle, WA 98195.

The premotor neurons involved in horizontal eye movements have been studied physiologically in detail in cats and monkeys, but very little is known about the anatomical distribution of these neurons in the monkey. We labeled the afferents to the abducens neurons by injecting horseradish peroxidase into one abducens nucleus in four monkeys and reacting the sections by the tetramethyl benzidine protocol.

Within the oculomotor complex, there were many small neurons labeled in the ventral and lateral margins of the contralateral medial rectus division. A small number of intermediate-size neurons were labeled in the middle gray of the contralateral superior colliculus. In the ipsilateral paramedian pontine reticular formation, there were a few intermediate-size cells labeled in the dorsal margin of the nucleus reticularis tegmenti pontis, greater numbers in the overlying medial nucleus reticularis pontis oralis, and still greater numbers in the dorsomedial nucleus reticularis pontis caudalis, rostral to the abducens nucleus. Labeled neurons were sprinkled lightly through the nucleus reticularis pontis caudalis ventral to the injected abducens nucleus. A compact group of labeled neurons occupied the dorsomedial reticular formation ventromedial and caudal to the contralateral abducens nucleus. Another dense focus of labeled cells was in the common margin of the nucleus prepositus hypoglossi and the medial vestibular nucleus, where they fuse. Smaller numbers of neurons occurred bilaterally, primarily contralaterally, in the nucleus prepositus hypoglossi. A third dense bilateral group of labeled cells was in the ventrolateral vestibular nucleus and the contiguous parts of the rostral pole of the medial vestibular nucleus. Bilaterally, sparsely distributed labeled cells extended caudally through the length of the medial vestibular nuclei. Small numbers of labeled cells were scattered bilaterally through the y-group.

The distributions of many of these individual populations are coextensive with groups of physiologically defined unit types, such as excitatory or inhibitory burst neurons, internuclear neurons, or "tonic-vestibular-pause" units. Having defined these premotor populations anatomically, we can begin to study their connections as well as their physiological characteristics.

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290.6 SMOOTH-PURSUIT-RELATED UNITS IN THE DORSOLATERAL PONS OF THE RHESUS MACAQUE. M. J. Mustari, A. F. Fuchs and J. Wallman. Reg. Primate Res. Ctr., Univ. of Washington, Seattle, WA 98195; City Univ. of N.Y., New York, NY 10031.

It is well established that the primate flocculus plays a role in the control of smooth-pursuit eye movements. Since the dorsolateral pons projects to the flocculus and receives input from cortical visual areas thought to participate in smooth pursuit, it may be a staging area for cortical smooth-pursuit signals destined for the flocculus. We recorded single-unit activity in the dorsolateral pontine nuclei of two rhesus macaques trained to make saccadic and smooth-pursuit eye movements in response to a moving target. To assess the visual sensitivity of these units, the monkeys were required to fixate a small spot while visual test stimuli were presented in their visual fields.

On the basis of their discharge during smooth pursuit of a small target spot and/or their response to a variety of visual stimuli, 50 single units were divided into three response types. Eye-movement-only units (24%) had a modulated discharge during smooth pursuit in the dark but not during saccadic tracking or fixation; in addition, they were not driven by full-field background movement or stroboscopic stimuli. These units were modulated over the full range of velocities tested (6.3-63°/s) although some units showed a peak in their velocity tuning curves. Eye-movement-and-visual units (54%) behaved like eye-movement-only units during smooth pursuit in the dark but also responded to some types of visual stimuli. The visual response generally included an on/off response (latency range 40-100 ms) and a direction-specific response to movement of either a full-field background ("Julesz" pattern) or a small test spot during fixation. The visual receptive fields were of either large ($D > 35^{o}$) or small ($D < 10^{o}$) diameter, included the fovea, and, in those tested, were binocular. Eye-movement-and-visual units were divided into those with the same (60%) and opposite (40%) eye-movement and visual direction preferences. Visual-only units (22%) had visual responses identical to those described for eye-movement-and-visual units; some also discharged during smooth pursuit of a small spot in the dark, but when the spot was turned off briefly (200-500 ms) and smooth pursuit continued with no visual target, the neuronal response dropped to its resting rate. Eye-movement-only units in a similar experiment continued to discharge after the spot had disappeared.

Our findings support the notion that the dorsolateral pons may provide the flocculus with a variety of visual and eye-movement signals that could be instrumental in the control of smooth-pursuit eye movements.

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290.7 FUNCTIONAL PROPERTIES OF BRAINSTEM CELLS INHIBITED FROM THE CEREBELLAR FLOCCULUS IN MONKEY. S.G. Lisberger and T.A. Pavelko*, Dept. Physiology and Div. Neurobiology, Univ. California, San Francisco, CA 94143

The primate flocculus provides signals for the control of horizontal smooth pursuit eye movements, and is necessary for long-term adaptive changes in the vestibulo-ocular reflex (VOR). It is known that the flocculus projects to the brainstem where it inhibits interneurons in the short latency VOR pathways. However, previous work has not revealed which functional class(es) of cells are the targets of floccular inhibition.

We recorded the activity of 140 cells in the brainstem of 2 monkeys. Each cell was characterized by its response to electrical stimulation of the flocculus as well as its firing in relation to eye movement and horizontal sinusoidal vestibular stimulation. Stimulating electrodes had been implanted in the flocculus at a site that induced ipsilateral smooth eye velocity: single shocks caused a twitch with an amplitude of 3-6°/s and a latency of 10 ms.

Single shocks to the flocculus inhibited a small group of cells (n=13) in the rostral pole of the medial vestibular nucleus. Latency ranged from 1.2 to 1.9 ms. With the head stationary, these cells fired steadily in relation to eye position with sensitivity averaging 1.94 spikes/s/° and emitted a burst of spikes just prior to saccades in their preferred direction. In most of the cells, the preferred direction was contralateral. For a given eye position the cells showed more variability in interspike intervals than is commonly found among brainstem cells that fire in relation to eye movements. During either smooth pursuit or the VOR at 0.2 Hz, ±10° firing rate was modulated sinusoidally and led eye position by an average of 40° (SD ±4.4°). When the monkey tracked a target that moved with him during head rotation (VOR cancellation), many cells retained a weak modulation of firing rate that could even be out of phase with the firing seen during the VOR. In contrast, cells in the abducens nucleus had phase leads averaging 17° (SD ±4.2°) during the VOR at 0.2 Hz, ±10° and showed no residual modulation during VOR cancellation.

In summary, we have found a group of eye movement cells that are inhibited from the flocculus and can be recognized by the quantitative relationship between their firing and eye movement. These cells may be a subgroup of those reported by Keller and Kamath to have disynaptic inputs from the vestibular nerve. (Supported by EY03878 and the McKnight and Sloan Foundations).

290.8 ACTIVITY OF MESENCEPHALIC CONVERGENCE CELLS DURING VERGENCE ADAPTATION. C. A. Tello* and L. E. Mays. Dept. of Physiological Optics and the Neurosciences Program, Univ. of Alabama in Birmingham. Birmingham, AL 35294.

Psychophysical experiments suggest that vergence eye movements are controlled by two subsystems: a rapid one which is the responsible for immediate, initial vergence movements; and a slower mechanism which is responsible for the tonic level of convergence. The fast mechanism is usually modeled as an integrator with a time constant of a few seconds. It has been proposed that the slow mechanism is also an integrator with a much longer time constant and is driven by the output of the fast integrator. Thus, the slow integrator can be slowly charged by forcing the eyes to converge to fuse binocular targets. The result is a relatively long lasting increase in the phoria, or resting vergence angle.

We studied the effect of vergence adaptation on the activity of mesencephalic convergence cells in 3 trained rhesus monkeys. Convergence cells have a firing rate which is a linear function of the vergence angle, without regard to the position of either eye in the orbit. Ordinarily, the activity of these cells is a reliable indicator of vergence angle. The monkeys viewed targets presented stereoscopically. In the unadapted condition, the animals were required to converge their eyes to different vergence angles for short periods (1-2 sec). Later, convergence adaptation was induced by gradually increasing the target vergence up to 10° over a period of 20-30 min, or until a relatively stable increase in esophoria was observed.

In both adapted and unadapted conditions there was a linear relationship between the firing rate of convergence neurons and vergence angle. For the majority of cells, the firing rate in the adapted state was consistently lower than in the unadapted condition for a given vergence angle. The magnitude of this decrease was roughly equal to the change in the phoria. These results suggest that many convergence cells behave as the output of a fast fusional integrator, and that this output is combined with that of a slow integrator to determine the vergence angle. (Supported by EY03463 and EY03039).

290.9 THE MESENCEPHALIC RETICULAR FORMATION AND SACCADIC EYE MOVEMENTS: A STIMULATION AND CHRONIC SINGLE UNIT STUDY. M. Gerez* and D.L. Sparks. Neurosciences Program, Dept. of Psychology, and Dept. of Physiology and Biophysics, University of Alabama in Birmingham, Birmingham, AL 35294.

Neurons that discharge before saccadic eye movements have been isolated in the mesencephalic reticular formation (MRF) and microstimulation in this region produces saccadic eye movements (see: Cohen et al., Doc. Ophthalmo. 1982. 4:325-335). Since the deeper layers of the superior colliculus (SC) project to the MRF, this study was designed to compare collicular and MRF patterns of saccade-related unit activity and stimulation effects.

Two rhesus monkeys with implanted eye coils were trained on fixation and saccade tasks. Standard chronic microelectrode recording and microstimulation methods were employed.

Stimulation of the MRF, extending 7-8 mm rostrally from the SC and over depths ranging from 3-8 mm, produced saccadic eye movements. In a single penetration, mixture of vertical, oblique and horizontal saccades was produced. An orderly progression of saccade trajectories, with gradual changes in direction and amplitude was usually observed. Microelectrode recordings indicated that the stimulated regions contained neurons, not merely fibers of passage, and that these neurons possessed collicular-like movement fields.

Stimulation along a single penetration produced different effects at different depths. Superficially, the size and direction of the saccade were largely independent of stimulation parameters and position of the eye in the orbit. At deeper sites, the orbital position during fixation influenced the size and/or the direction of the movement. The amplitude of stimulation-induced saccades was reduced as the fixation point approached a particular area of the visual field. Stimulation while fixating a point in this area failed to produce a saccade.

Current models of the saccadic system (Robinson, Ann. Rev. Neurosci. 1981. 4:463-503) require, as an input to brainstem circuitry, a signal of the desired position of the eye in the orbit. A possible role for the MRF in the formation of this signal is suggested by our results.

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290.10 RESPONSE OF MIDBRAIN NEURONS IN THE ALERT CAT DURING OPTOKINETIC STIMULATION. M. LeTaillanter* and J.H. Anderson (SPON: R.E. Poppele). Depts. of Otol. and Phys., Univ. of Minn., Minneapolis, MN 55455

Single unit recordings in the monkey have provided much evidence that the mesencephalic reticular formation (MRF), in the region of the interstitial nucleus of Cajal (INC) (King et al, J Neurophysiol, 46:549, 1981) and areas more rostral to it (Büttner et al, Br. Res., 130:239, 1977), are important for generating vertical saccades and maintaining fixation. Cats with kainic acid lesions of INC showed deficits in the vertical VOR (Anderson et al, Neuroscience Lett., 14:259, 1979; King and Leigh, Functional Basis of Ocular Motility Disorders, 267, 1982) which suggested that this area of the midbrain may be involved with the integration of an eye velocity signal. To provide further evidence for this, we have recorded the response of single units in the alert cat during spontaneous eye movements and during vertical optokinetic and optokinetic afternystagmus.

Alert cats were positioned on their sides and placed inside an optokinetic drum. Forty-five single units were recorded and classified as burst, burst-tonic, or tonic on the basis of their response during spontaneous eye movements. Eleven units increased their firing for upward movements and 34 for downward movements. Among the up units, 5 were burst, 3 burst-tonic, and 3 tonic; among the down units, there were 9, 6, and 19 units respectively. For the burst and burst-tonic units the latency before saccade onset was -2 to 17 m sec (mean 2.9) for up units, and 0-30 m sec (mean 14.2) for down units. During optokinetic and optokinetic afternystagmus (OKAN) the units had the same latencies and responded in the same way, with regard to their burst or tonic behavior, as during spontaneous eye movements.

The presence of tonic units in the MRF and their response during OKAN shows that these units carry an eye position signal and support the idea that the region of INC in the mesencephalic reticular formation participates in the integration of an eye velocity signal.

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290.11 VISUO-MOTOR UNITS IN FRONTAL DORSOMEDIAL CORTEX OF MONKEY. J. Schlag and M. Schlag-Rey. Dept. of Anatomy and BRI, UCLA, Los Angeles, CA 90024.

Microelectrode recording and stimulation were carried on in order to locate and study a neural population concerned with oculomotricity on the medial frontal cortex of monkey, as suggested by Woolsey's mapping of the supplementary motor area. Three monkeys (macaca nemestrina) were trained in visual tasks providing instances of visually triggered saccades, saccades away from target, saccades of retargeting, steady fixation, pursuit, as well as spontaneous saccades between trials. Eye position was recorded with the scleral search coil technique.

Units active with eye movements and units responding to visual stimuli were found in close proximity within the dorsal medial edge of frontal cortex. All saccade-related units had a preferred direction. Those which were presaccadic had a long lead (e.g. 500 ms), with a time course reminiscent of the readiness (Bereitschaft) potential. The lead did not terminate in a burst. Some of these units were active with spontaneous saccades in darkness. Others required the presence of a visual target; among those, some discharged only when a saccade in the preferred direction was made toward the target, even when looking away was a condition for reward. Microstimulation at the site of recording usually evoked saccades in the cell preferred direction with 120 ms trains and currents of 6-50 μA. Pause units and eye-position units were also encountered but more rarely. Visual responses were phasic or tonic. Different units gave phasic responses to (a) onset and retargeting of stimuli, (b) onset, retargeting, and offset of stimuli, (c) offset of stimuli. Depending on the cell, tonic responses consisted in increases or decreases of firing, and they occurred with stimulus fixation and pursuit.

The presence of an oculomotor region in frontal dorsomedial cortex corresponds to findings in cat. In monkey, the region explored is known to have relations with the frontal eye field and superior colliculus. Its role needs to be specified, particularly with respect to the supplementary motor area and the prearcuate eye field.

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290.12 ROLE OF THE OCULOMOTOR SYSTEM IN BLINKING. C. Evinger. Dept. Neurobiology & Behavior, SUNY Stony Brook, Stony Brook, NY 11794

The oculomotor system figures prominently in blinking. Each blink requires inhibition of levator palpebrae motoneurons to eliminate the upward pull on the eyelid, and excitation of other oculomotor motoneurons, to create a small, upward eye rotation. Since humans blink nearly 19,000 times a day, blinking is a constant activity of the oculomotor system. Nevertheless, little is known about the behavior of oculomotor motoneurons with blinking or the integration of blinking with other oculomotor activities.

To determine the pattern of extraocular muscle activation during lid closure, EMG electrodes were implanted in the eye muscles of rabbits and pigeons. With both reflex (rabbit) and spontaneous blinks (pigeon), antagonsitic pairs of extraocular muscles showed simultaneous bursts of activity. Likewise, all antidromically identified oculomotor motoneurons recorded in the alert rabbit produced a transient increase in activity with each reflex blink. Thus, during a blink, cocontraction of the extraocular muscles retracted the eye into the orbit and secondarily produced an upward eye rotation.

Since blinking excites antagonistic pairs of extraocular motoneurons, any eye movement needing precise balancing of motoneuron activity requires a suppression of blinking. To test this, spontaneous blinking was monitored as humans tracked a target moving horizontally at 5 deg/sec for 6 or 12 sec followed by a 15 sec period of fixation. A nearly complete cessation of blinking accompanied the smooth pursuit eye movements, but fixation initiated a period of intense blinking in which the blink rate slowly returned to normal. Thus, smooth pursuit eye movements necessitated a suppression of spontaneous blinking.

Blinks often accompany rapid head and eye movements. To study this synkinesis, humans were asked to fixate a target stepping horizontally 30 or 60 deg using their eyes alone or a combined eye and head movement. Blinks tended to occur with saccadic eye movements but almost every head movement had a concomitant blink. Similarly, pigeons blinked with every head movement. Since the lid movement began before the head movement, a sensory signal cannot account for the blink. Thus, blinks and head movements were programmed concurrently.

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290.13 OPIATE RECEPTORS: CHARACTERIZATION IN THE AVIAN CILIARY GANGLION. Jonathan T. Erichsen, Kent T. Keyser,* R. Suzanne Zukin and Harvey J. Karten. Departments of Neurobiology & Behavior and Psychiatry, SUNY at Stony Brook, NY 11794; Departments of Biochemistry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

Enkephalin-like immunoreactivity has been localized in preganglionic terminals of the avian ciliary ganglion. Radioimmunoassay and HPLC studies have shown that both leu- and met-enkephalin are found in the ganglion. The presence of enkephalins suggests that opioid peptides may play a role in the transmission of information to the ciliary ganglion. Such a role would require an appropriate receptor. Receptor binding techniques were used to assay for the presence of opiate receptors in the ganglion, as well as to identify the different subtypes and their relative abundance.

Pigeons were decapitated and the ciliary ganglia were quickly removed on ice under a dissecting microscope and placed in ice cold saline. Ganglia from 10-20 animals were pooled, homogenized and used in binding assays. Tritiated dihydromorphine (^{3}H-DHM) binding to ciliary ganglia homogenates was saturable, of high affinity and reversible. Scatchard analysis of ^{3}H-DHM binding revealed an apparent single class of binding sites (K_d =8.8nM, B_{max}=260fmol/mg protein). Mu opiate receptor binding was assayed by measuring the ability of D-Ala^2,N-Phe^4-Gly-ol^5- enkephalin (DAGO) to displace ^{3}H-DHM binding. Delta receptor binding was assayed by the ability of D-Ala^2,D-Leu^5-enkephalin (DADLE) to displace ^{3}H-DHM binding. IC_{50} values of 45±5nM and 10±2nM were determined for DAGO and DADLE, respectively.

These data support the presence of high affinity opiate binding sites in the ciliary ganglion; the predominant type would appear to be the delta receptor. This finding is consistent with the emerging view that the delta receptor is the most common subtype in non-mammalian vertebrates. In other cases, the mu receptor appears to be morphine-selective whereas enkephalinergic systems are often associated with delta receptors, which are much more sensitive to enkephalin than to morphine. The miotic effect of systemically introduced morphine is well documented in a number of vertebrates (e.g. dogs and primates). Our data would predict that the pigeon's pupillary response would exhibit a greater sensitivity to enkephalin than to morphine. Experiments are currently underway to investigate the effects of a number of opiate ligands on pupil size in the pigeon. Supported by EY04587 (JTE), EY04796 (HJK) and DA01843 (RSZ).

290.14 FINE AND GRANULAR MUSCLE FIBERS OF THE HUMAN EXTRAOCULAR MUSCLES ARE MULTIPLY INNERVATED BY EN GRAPPE ENDINGS. M. Sadeh*, L.Z. Stern, Department of Internal Medicine (Neurology), Univ. of Arizona Health Sciences Center, Tucson, AZ 85724.

Studies correlating the type of end-plate with muscle fiber type in animal and human extraocular muscles (EOM) (Hess, J.Cell.Comp.Physiol.58:63,1961; Dietert, Invest. Ophthal.4:51,1965; Durston, Brit.J.Ophthal.58:193,1974; Ringel et al., Arch.Ophthal.96:1067, 1978) have suggested that the coarse muscle fibers (*felderstruktur*) are multiply innervated by *en grappe* endings and the fine and granular fibers (*fibrillenstruktur*) are singly innervated by *en plaque* endings. We have reexamined the question of which fiber type is multiply innervated using a comprehensive histological and histochemical approach.

The EOM were removed during postmortem examination from 4 adults without ocular or neuromuscular disease. Longitudinal sections were stained with bromoindoxyl acetate for end-plate cholinesterase followed by silver-gold impregnation for nerves. Longitudinal and serial transverse sections were stained with H&E, the modified Gomori trichrome, NADH-TR, ATPase at pH 9.4 and after preincubation at pH 4.2 and nonspecific esterase. Additional sections were stained for end-plate cholinesterase combined with ATPase 9.4 and 4.2. Study of transverse serial sections showed that the small *en grappe* endings occurred multiply on the fine and granular fibers and the large *en plaque* endings singly on the coarse fibers. This observation was confirmed studying the sections stained for cholinesterase and ATPase 9.4 and 4.2. Nonspecific esterase stain showed that all darkly stained fine fibers have multiple end plates. Serial longitudinal sections from the periphery to the center of the muscle revealed that most of the peripheral fibers had a single *en plaque* end plate and since over 85% of the peripheral fibers are coarse, this further suggests that the coarse fibers are singly innervated.

Our conclusions contradict previous reports and may provide a new basis for further studying morphological, metabolic and functional correlations in human EOM.

291.1 DIFFERENTIAL EFFECTS OF CHOLINERGIC AND GABAERGIC ANTAGONISTS ON SPATIAL FREQUENCY CHANNELS IN THE RAT VISUAL SYSTEM. D.A. Fox. Coll. of Optometry, U. Houston, Houston, TX 77004.

The channel model of spatial vision states that the sine-wave components of the retinal image are transferred to the visual cortex via a number of separate neural channels. A pharmacological study with physostigmine, an AChE inhibitor, suggested there were two (high and low) spatial frequency components in the cat pattern-reversal evoked cortical potential (PREP) (Science 221: 1076,1983). To examine the pharmacological selectivity and spatial frequency specificity of the neural channels, dose-response experiments were performed in Long-Evans hooded rats with a cholinergic muscarinic (scopolamine) or gabaergic (bicuculline) receptor antagonist. Contrast sensitivity functions (CSF) were determined in chronically implanted awake rats under two conditions, predrug or control and drug, using PREP methodology. Preliminary results revealed that both drugs decreased spatial resolution in the rat. In order to compare the relative contribution of the cholinergic muscarinic and gabaergic systems on spatial frequency channels, drugs were administered in three separate (ip) doses with each drug dose matched to produce similar decreases in visual acuity. CSFs in controls (N=13) were characterized by a peak at 0.2 cycles/degree (cpd), a high spatial frequency cut-off at 1.4 cpd and a low spatial frequency roll-off at approx. 0.1 cpd. Bicuculline administration (N=10/dose) resulted in a relatively large, non-specific decrease in the CSF at all spatial frequencies. Mean sensitivity loss (dB) of the CSF was 4.5, 8.6 and 13.0 for low, med. and high doses, respectively. In contrast, scopolamine administration (N=12/dose) resulted in a relatively small decrease in the low spatial frequency limb (0.1-0.4 cpd) of the CSF and a relatively large, specific decrease in the high spatial frequency limb (0.5 cpd and above) of the CSF. The decrease in the latter limb was of similar magnitude to that observed for bicuculline at all spatial frequencies. For low, med. and high doses of scopolamine mean sensitivity loss (dB) was 1.8, 3.8 and 6.8, respectively. At 0.5 cpd and above, mean sensitivity loss (dB) was 4.1, 8.5 and 12.2 for the same three doses. The non-specific bicuculline results do not at present help differentiate specific spatial frequency channels in the rat visual system, however, they are consistent with its role as an inhibitory transmitter at all levels of the visual system. The differential spatial frequency results with scopolamine demonstrate that spatial vision in the rat is mediated by at least two different detector channels. Supported by NIEHS Grant ES 03183.

291.2 THE EFFECT OF MONOAMINES ON NEURONES OF THE LATERAL GENICULATE NUCLEUS IN VITRO. V. Crunelli*, N. Leresche*, J.A. Kemp*, M. Pirchio* and J.S. Kelly. Department of Pharmacology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, U.K.

Intracellular recordings were made from neurones of the dorsal and ventral lateral geniculate nucleus (LGN) of the rat in vitro (300 μm thick slices). These neurones had resting membrane potentials of -50 to -70mV and input resistances, calculated in the linear portion of voltage-current plots, of 26 to 45 MΩ. Intracellular injection of horseradish peroxidase or Lucifer Yellow showed impaled neurones of both dorsal and ventral LGN to possess the morphological features of thalamic relay cells rather than interneurones.

In neurones held at potentials more negative than -65mV a pulse of depolarizing current or stimulation of the optic nerve evoked a Ca^{2+} dependent spike resistant to TTX and abolished in the presence of Co^{2+} or Ca^{2+} free medium. However, such Ca^{2+} potentials were only observed in neurones of the dorsal LGN and were absent in cells of the ventral portion of the nucleus.

Although in both dorsal and ventral neurones small iontophoretic applications of serotonin (5HT) and noradrenaline (NA) had no effect on resting membrane potential, input resistance or on the epsp evoked by stimulation of the optic nerve, in neurones of the dorsal LGN the Ca^{2+} spikes were reduced by 5HT and increased by NA. The depolarizing response of dorsal LGN neurones to glutamate was also reduced by 5HT and potentiated by NA. This effect of the monamines on the glutamate evoked response was unaffected by TTX and blocked by Co^{2+}.

These results show the monoamines to have a potent action on the voltage sensitive Ca^{2+} mediated spikes of dorsal LGN neurones. Thus in vitro only neurones at potentials more negative than -65 mV in the dorsal LGN can indeed be modulated by the monoamines.

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291.3 VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN THE RABBIT RETINA: LOCALIZATION, FUNCTION AND DEVELOPMENT. N.X. Chen*+, K.R. Fry, J.A. Pachter* and D.M.K. Lam, Program in Neuroscience and Cullen Eye Institute, Baylor College of Medicine, Houston, TX 77030 and Zhongshan Medical College, Guangzhou, China+

In the rabbit retina, VIP stimulates cAMP formation while VIP-immunoreactive processes but not their somas have been demonstrated (Schorderet, Life Sci. 20:1741, Tornqvist et al. Histochem. 76:137). For a better understanding of the role of VIP in retinal function and development, we have identified the VIP-immunoreactive neurons in the rabbit retina, as well as characterized the stimulation of adenylate cyclase by VIP.

Immunofluorescent studies were performed in retinas of adult and postnatal rabbits, which had been injected intraocularly with colchicine 18-24 hrs prior to sacrifice, using a polyclonal VIP antibody from Immuno-Nuclear Corporation. Strongly fluorescent amacrine cell bodies were predominantly located along the vitreal border of the inner nuclear layer. Processes of these cells ramify in sublaminas 1,3 and 5 of the inner plexiform layer. In developing retinas labelled processes were observed at approximately 6 days postnatal. By 17 days after birth VIP-immunoreactive amacrine cells appeared mature morphologically.

These results, together with our previous study on the retinal VIP system (Pachter and Lam, Soc. Neurosci. Abst., 1983), suggest that in the rabbit retina, (1) the stimulation of cAMP formation by VIP occurs in the inner plexiform layer and (2) the emergence and maturation of VIP immunoreactive neurons follow a similar developmental time course as those for VIP-stimulated adenylate cyclase.

This work was supported by the Alberta Heritage Foundation (KRF), NIH (EY02423 and EY02608) and Retina Research Foundation (Houston).

291.4 AROMATIC AMINO ACID METABOLISM IN RAT RETINA. C.J. Gibson. Department of Pathology, University of Western Ontario, London, ONT N6A 5C1.

Dopamine (DA) and melatonin (MEL) are two bioactive amine synthesized in retina, which may play a role in regulating light sensitivity and visual function. These two compounds are derived from the dietary amino acids, tyrosine (TYR) and tryptophan (TRP), respectively. Peripheral administration of l-tyrosine (100 mg/kg) raises retinal TYR and, in light-activated retina in which the activity of the rate-limiting enzyme, tyrosine hydroxylase (TH), is high, increases DA turnover (Gibson, Watkins & Wurtman, J Neural Trans 56:153, 1983). In contrast, DA turnover is significantly enhanced in both light and dark-exposed retina following l-dihydroxyphenylalanine (DOPA) administration (the hydroxylated product of TYR and immediate amino acid precursor of DA). Male Sprague-Dawley rats were kept in the dark for 14 hours or exposed to light for 2 hours and injected with DOPA (100 mg/kg) or its vehicle. All animals were sacrificed one hour later and retinae were analyzed by HPLC for DOPA, DA and dihydroxyphenylacetic acid (DOPAC).

			DA (ng/pair of retina)	DOPAC
DARK	Vehicle	(n=8)	8.4 ± 0.6	2.9 ± 0.1
	DOPA	(n=7)	13.0 ± 2.0*	22.1 ± 6.9**
LIGHT	Vehicle	(n=13)	10.0 ± 0.7	5.9 ± 0.4
	DOPA	(n=9)	12.8 ± 1.4*	29.2 ± 4.1**

*p<0.01 and **p<0.001 differs from vehicle

Exposure to light enhanced DA turnover, increasing both DA and DOPAC level. After DOPA injection, DOPAC increased 5 to 7-fold in both light and dark-adapted rat retina. MEL synthetic enzymes also undergo a daily rhythm with greatest activity in the dark phase of the cycle. Administration of TRP (100 mg/kg) one hour prior to sacrifice increased retinal MEL level significantly at 10 PM (DARK), from 233 to 562 pg/pair of retina, and only slightly in rats sacrificed at 10 AM (LIGHT), from 110 to 150 pg/pair, when synthesis of MEL is low. Administration of the aromatic amino acid precursors increases retinal DA and MEL turnover and may be useful in selectively manipulating these compounds and hence altering visual function. These studies were supported by grants from the NEI (#RO3 EY04669-01) and Human Nutrition Research Council of Ontario.

291.5 QUANTITATIVE DISTRIBUTION OF ASPARTATE AMINOTRANSFERASE ACTIVITY IN COCHLEAR NUCLEUS, OLFACTORY BULB AND RETINA OF ALBINO RATS. Donald A. Godfrey and C. David Ross, Dept. of Physiology, Oral Roberts Univ., Tulsa, OK 74171

The enzyme aspartate aminotransferase (AAT), has been proposed as a possible indicator of neurons using Glu and/or Asp as transmitter. Further, Glu and/or Asp have been proposed as possible neurotransmitters in early stages of sensory information processing, in the cochlear nucleus (CN), olfactory bulb (OB), and retina. Quantitative histochemical procedures were used to map the distributions of AAT activity in these sensory structures and compare them with the distributions of Glu and Asp as well as of the citric acid cycle enzyme malate dehydrogenase (MDH). Enzyme activities are expressed as mol/kg dry wt/hr at 37°C (AAT) or 25°C (MDH). Average AAT and MDH data are presented from 5 rats for CN and OB, 7-12 for retina. Standard errors were typically about 10% of the mean values. Ratios of AAT activity to Glu and Asp data (mmol/kg dry wt) from our previous work on CN and OB of rat, but from monkey retina (Berger et al., J. Neurochem. 28:159-163, 1977), are also presented.

		AAT	MDH	AAT/MDH	AAT/Glu	AAT/Asp
CN	Dorsal, molecular	39.3	23.8	1.65	1.02	2.93
	Dorsal, fusiform	31.8	20.2	1.57	0.95	2.09
	Granular	26.6	18.6	1.43	0.69	1.97
	Posteroventral	26.2	17.0	1.54	1.11	2.17
	Anteroventr, rostr	29.2	18.2	1.60	1.24	1.73
OB	Fiber	21.0	14.5	1.45	0.58	2.80
	Glomerular	31.6	22.1	1.43	0.90	3.29
	Ext plex, superf	46.6	27.2	1.71	1.08	2.88
	Ext plex, deep	44.6	27.0	1.65	1.03	2.35
	Mitral	41.2	25.2	1.63	0.91	2.23
	Int plex	39.2	23.8	1.65	1.02	2.78
	Granular	29.1	19.3	1.51	0.75	2.75
Retina	Outer segm	11.9	4.0	2.98	0.74	1.59
	Inner segm	63.2	15.8	4.00	1.71	7.52
	Outer nucl	17.0	7.3	2.33	0.45	1.31
	Outer plex	37.0	14.6	2.53	0.97	4.11
	Inner nucl	29.9	13.3	2.25	0.69	3.48
	Inner plex	41.5	18.6	2.23	0.73	3.14
	Ganglion cell	23.0	14.0	1.64	0.25	0.84

The best correlation between the distribution of AAT activity and that of an amino acid concentration was with Asp in OB (r=0.92). The closest correlation in all three structures was between the distributions of AAT and MDH activities (r=0.97 in CN, 0.98 in OB, and 0.77 in retina). (Supported by NIH grants NS17176 and EY03838.)

291.6 IONTOPHORETIC APPLICATION OF TRANSMITTER CANDIDATES AND SINGLE UNIT ANALYSIS IN THE AFFERENT SYNAPSE OF THE ACOUSTICO-LATERALIS RECEPTORS OF PLOTOSUS. T.Nagai*, S.Obara* and N.Kawai*. (SPON: R.M. Bradley). Dept. Physiol., Teikyo Univ. Sch. Med., Tokyo 173; and (N.K.) Dept. Neurobiol., Tokyo Met. Inst. for Neurosci., Tokyo 183, Japan.

In an attempt to identify the transmitter in acousticolateralis receptors, effects of acidic amino acids were examined in the in situ ampullary electroreceptors of the marine catfish Plotosus. The ampulla (sensory epithelium) was hyperpolarized by 5 mV to suppress receptor cell activities, continual transmitter release, and hence the "resting" afferent discharges. To the suppressed ampulla, i.e. in the absence of transmitter, amino acids were applied by iontophoresis through multi-barrelled electrodes. Evoked single unit responses were analysed by instantaneous frequency (F) records. L-glutamate (Glu) postsynaptically induced single unit responses (Glu response), with F records in a gradual time course. Postsynaptic afferent responses also could be induced by passing sinusoidal current, through an elctrode at the nerve trunck. These evoked responses were quite similar in time course to the applied current, suggesting that F records would show a truncated slow potential in the afferent terminals.

At the Glu sensitive spots, kainate (KA) and quisqualate (QA) induced more persistant, and L-homo-cysteate (HCA) weaker, afferent excitation than Glu, with similar latency. L-aspartate (Asp) induced small and slow responses with a long latency. D-Glu, D-Asp, L-cysteate (CA), L-cysteine sulfinate (CSA) and N-methyl-DL-aspartate (NMA) generally induced no response. Several aspartate analogues, L- and D-Asp, CA and CSA, when applied as a conditioning pulse to Glu, exerted prolonged potentiation on the Glu responses. Asp also potentiated the responses to KA and HCA.

Responses to the transmitter (focal response) were induced by focal stimulation of a few receptor cells at the Glu sensitive spot, through a NaCl barrel of the electrodes. The focal response consisted of a fast initial peak followed by a marked decline, which is quite in contrast to the Glu responses. A conditioning Asp pulse had virtually no effect on the focal response. The Glu response and focal response were further studied with Joro toxin (JSTX), a Glu antagonist in lobster neuromuscular junction. JSTX was added to the bath perfusing the ampulla. Responses to Glu, as well as KA and QA, were abolished irreversibly depending on JSTX doses, while the focal responses and the resting discharges were little affected. The differential response properties of the Glu and focal response clearly refute the Glu hypothesis in the acousticolateralis receptors.

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291.7 RETROGRADE TRANSPORT OF [^{3}H]-D-ASPARTATE (D-ASP) IN THE COCHLEAR NERVE OF THE CHICKEN. Lyman, D.*, Book, K.*, and Morest, D.K., Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06032

D-ASP is a metabolically inert analogue of L-glutamate and/or L-aspartate (L-GLU/L-ASP) which can specifically label by retrograde transport those neurons using L-GLU/L-ASP as transmitters. For example, spiral ganglion cells are labeled in the cat and guinea pig by injecting D-ASP in the cochlear nucleus (Oliver et al, J Neurosci '83). We used D-ASP to investigate whether L-GLU/L-ASP could be transmitters in avian cochlear ganglion cells, in preparation for developmental studies.

Unilateral injections of 3 mM D-ASP (0.5-1.0 µl; specific activity, 20 Ci/mmol) were made in nuc magnocellularis (NMC) of white Leghorn hens, following exposure of the 4th ventricle. After 0.2, 3, 6, 19, and 23 hrs, the birds were perfused with aldehydes and serial sections of the brain stems (25 µm) and cochleas (2 µm) were collected and developed for autoradiography. Injection sites were defined by heavy labeling of glial cells, which presumably mediate some of the high-affinity uptake of D-ASP.

In all cases one entire NMC was included in the injection site, which encroached to varying extents ipsilaterally on neighboring nuclei. Around the injection site a zone of lower grain density extended to the CNS-PNS boundary of the 8th nerve. Peripheral to this, grain density decreased abruptly, but it remained above background. Labeled neuronal somata and their proximal processes were seen in the ipsilateral cochlear ganglion in all cases; the distal processes and basilar papilla did not seem to be labeled significantly, even at the longest exposure time examined (64 d). Label was not seen in the contralateral ganglion or basilar papilla.

In the ipsilateral ganglion heavily labeled neurons and axons appeared at the shortest survival, while glia were unlabeled. By 23 hrs, ganglion cell label decreased, but some heavily labeled satellite cells were seen. Thus a transfer of label from neuronal to glial compartments may have occurred. A proximal-to-distal decrease in the density of neuronal label was detected in several ganglia.

These results provide evidence for the uptake of D-ASP by cochlear nerve fibers in NMC and its retrograde transport to the cochlea. Hence L-GLU/L-ASP may be transmitters in the avian cochlear nerve.

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291.8 THE EFFECT OF UNILATERAL BASILAR PAPILLA REMOVAL ON THE DISTRIBUTION OF ACETYLCHOLINESTERASE IN CANARY AUDITORY NUCLEI. J.M. Greenspon*,[1], B. Fass[2] and J. Humpal*,[3] (SPON: R.M. Beckstead[2]). 1) Psychol. Dept., Hobart & Wm. Smith Coll. Geneva, NY 14456. 2) Neurosurg. Dept., Univ. Virginia Sch. Med., Charlottesville, VA 22908. 3) Psychol. Dept., Johns Hopkins Univ., Baltimore, MD 21218.

The acquisition of birdsong is dependent on such variables as genetic factors, sensitive periods, hormonal actions on brain, and auditory experience. Of specific interest to our research is that male (Waser and Marler, 1977) and female (Greenspon, 1983) canaries learn songs from auditory information. Therefore, an understanding of the avian brainstem auditory system (ABAS) is important for investigating the neural control of song.

Previous research on the ABAS has studied its cytoarchitecture, connectivity, and synaptology mostly using non-passeriformes. For example, the anatomy of the ABAS in chickens consists of the VIIIth nerve innervating n. angularis (NA) and n. magnocellularis (NM) ipsilaterally. NM, in turn, projects bilaterally to n. laminaris (NL; Parks, and Rubel, 1978). However, virtually nothing is known about the neurotransmitters utilized by the ABAS. Thus we investigated the distribution of acetylcholinesterase (AChE) in NA, NM, and NL of five intact canaries. Since the somata of NM neurons stained intensely for AChE in the intact birds we processed three additional cases to determine whether input to NM, other than that from the VIIIth nerve, might account for the intense staining of NM somata. These later cases sustained unilateral removal of their basilar papilla followed by a 31 d. survival time. All tissue was processed according to the method of Naik (1963) and included control material processed for nonspecific cholinesterases. Control material exhibited little or no staining.

All brains were rated by two experimenters who determined independently whether the somata and/or neuropil of each nucleus stained positively for AChE using the following scale: intense; heavy; moderate; light; and weak. Despite noticeable morphological changes in the deafferented NM there were no noticeable differences in the distribution of AChE between groups. Neuronal somata stained intensely in NA and NM while only staining lightly-to-moderately in NL. Neuropil of NA and NL stained positively; the former more intensely than the latter. Neurons in NA and NM appear very rich in AChE, whereas neurons in NL do not. Furthermore, the apparent richness of AChE in NA and NM neurons may be attributable to afferents other than those from the VIIIth nerve.

291.9 MUSCARINIC RECEPTOR LOCALIZATION IN RABBIT CAROTID BODY. B.G. Dinger*, T. Hirano* and S.J. Fidone, Dept. of Physiology, Univ. of Utah Sch. of Med., Salt Lake City, UT 84108.

It has been reported that the specific cells (glomus, Type I) in the mammalian carotid body store and release cholinergic substances in response to natural stimuli (hypoxia, low pH, hypercapnia). Despite an abundance of pharmacological data, fundamental questions remain unanswered concerning the chemoreceptive action of acetylcholine (ACh). Among the tenable hypotheses are those in which ACh serves as a transmitter of chemosensory information between Type I cells and synaptically apposed afferent terminals of the carotid sinus nerve (CSN). Alternatively, ACh may play a modulatory role by influencing various processes related to chemoreception (e.g., stimulus-induced release of catecholamines from glomus cells).

The present investigation set forth to characterize and localize muscarinic receptors in the carotid body. Equilibrium binding assays with ^{3}H-QNB determined a K_D of 57.3 pM and a B_{max} of 8.72 pmoles/gm of tissue. The order of potency of competing drugs was QNB>atropine>bethanechol>nicotine. Following chronic section of the CSN (12-15 days) the number of specific ^{3}H-QNB binding sites was unchanged. In contrast, specific binding declined by 44% 12-15 days following removal of the superior cervical ganglion, which supplies sympathetic fibers innervating primarily the vasculature of the carotid body. Comparative studies demonstrated a 51% lower concentration of ^{3}H-QNB binding sites in normal cat vs. rabbit carotid bodies.

The data suggest an absence of muscarinic receptors on chemosensory afferent fibers in the rabbit carotid body. Since our previous studies with ^{125}I-α-bungarotoxin (Brain Res. 205: 187-193, 1981) also suggested an absence of nicotinic sites on afferent nerve endings, ACh appears unable to act as either an excitatory (nicotinic) or inhibitory (muscarinic) neurotransmitter between Type I cells and afferent terminals. It does appear, however, that muscarinic (and nicotinic) receptors are associated with Type I cells and sympathetic axons. We are currently investigating the pharmacological effects of QNB on the stimulus evoked catecholamine release from Type I cells and on CSN activity. (Supported by USPHS Grants NS-12636 and NS-07938).

291.10 A RESTRICTED POPULATION OF VAGAL SENSORY NEURONS HAVE TWO-COMPONENT SPIKE AFTERHYPERPOLARIZATIONS. J. Fowler, D. Weinreich, and R. Greene*, Dept. of Pharmacology & Exp. Therapeutics, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Intracellular recordings from neurons of the rabbit nodose ganglion in vitro (23-25°C) revealed two types of afterhyperpolarizations (AHP) following single action potentials: a fast AHP (AHPf) lasting 149.6 msec (± 43.4; n=18) and a long-lasting (2.5 to 24 sec; n=26) and slowly developing AHP (AHPs). The AHPs was not found in A-cells and was present in about 50% of the C-cells examined. All neurons studied possessed an AHPf.

The AHPs was associated with a decrease in input impedance. Its duration and amplitude were proportional to the number of preceding spikes. At a pre-stimulus membrane potential of -55 mV, maximum AHPs amplitude was 11.1 mV (± 1.2; n=6). AHPs amplitude was increased in zero- and 0.1 x normal extracellular K^{+} and was reduced in 2 x normal K^{+}. The mean reversal potential for the AHPs was -78.8 mV (± 3.1; n=4). The amplitude of the AHPs was reduced in 0.1 x normal extracellular Ca^{++} (n=5). Calcium antagonists (Cd^{++}, Co^{++}, Ni^{++}) reduced or abolished the AHPs (n=6). In the presence of low calcium or calcium antagonists the AHPf was reduced (>50%) in 5/11 neurons.

Pressure-applied soluble extracts prepared from purified human lung mast cells abolished the AHPs without affecting the AHPf (n=14). Similarly pressure- or bath-applied prostaglandins (PGE_1, $PGF_{2\alpha}$; 1-10 µg/ml) reduced only the amplitude of the AHPs (n=9). Neurons responsive to mast cell extracts were unaffected by pressure-applied histamine at concentrations equal to or five-fold larger than that estimated to be present in soluble extracts.

These results indicate that a calcium-dependent potassium conductance underlies the AHPs. We suggest that the AHPs plays a role in the excitability of a subpopulation of C-fiber afferents and that the AHPs provides a substrate for a direct action of arachidonic acid metabolites released from lung mast cells.

291.11 SEROTONIN DECREASES THE CALCIUM-DEPENDENT COMPONENT OF ACTION POTENTIALS RECORDED FROM FROG DORSAL ROOT GANGLION CELLS. G.G. Holz, IV, S.A. Shefner and E.G. Anderson, Dept. Physiol., Tufts Univ. Schl. Med., Boston, MA 02111 and Dept. Physiol. and Biophys., and Dept. Pharmacol., Univ. Il. Col. Med., Univ. Illinois at Chicago, Chicago, IL 60612.

We examined the effects of serotonin (5-HT) on the Ca^{+2}-dependent component of action potentials recorded from frog dorsal root ganglion cells treated with 7.5 mM tetraethylammonium (TEA). Intracellular recordings were obtained from the somata of type A and C neurons as previously described (Holz et al., Soc. Neurosci. Abst. 9:254, 1983). TEA increased the duration of action potentials recorded from type A and C neurons by 241 ± 34% (S.E.M., n=31), an effect associated with the appearance of a plateau phase on the falling limb of the spike. The plateau phase results from calcium influx since it was reduced in amplitude and duration when calcim was omitted from the Ringer, and eliminated when the Ringer contained 2-4 mM manganese (n=7).

During treatment with 7.5 mM TEA, 5-HT (10 uM) decreased the duration of action potentials recorded from 16 of 22 type A neurons (mean decrease of 32 ± 4%) and 3 of 6 type C neurons (mean decrease of 33%). This decrease resulted from a narrowing of the plateau phase on the falling limb of the spike. The rate of rise and peak amplitude of the spike remained unchanged, but the amplitude and duration of the spike afterhyperpolarization was reduced, an effect which may be secondary to a decrease in voltage-dependent calcium influx. The response was concentration-dependent over a range of 10nM-10uM 5-HT, was rapid in onset and offset, and showed no tachyphylaxis to repeated or prolonged applications of 5-HT. The decrease in spike duration was not associated with a change in resting membrane potential or input resistance when the concentration of 5-HT was 1 uM or less, but was accompanied by a slow 4-8 mV depolarization recorded from 33% of type A neurons during exposure to 10 uM 5-HT. This depolarization was associated with an underlying increase in input resistance which was masked by rectification. In the remaining 67% of the type A neurons tested, 10 uM 5-HT reduced the action potential duration without affecting membrane potential or input resistance. These findings demonstrate a selective inhibitory action of low concentrations of 5-HT on voltage-dependent calcium influx, an effect independent of the change in membrane potential produced by higher concentrations of 5-HT.

291.12 SURFACE ANTIGENS ON FUNCTIONAL SUBSETS OF PRIMARY SENSORY NEURONS. J.Dodd and T.M.Jessell, Dept. of Neurobiology, Harvard Medical School, Boston MA 02115.

Subpopulations of dorsal root ganglion (DRG) neurons can be distinguished on the basis of their peripheral receptive properties, spinal terminal arbors and neuropeptide content. Surface molecules expressed by functional subsets of DRG neurons have not yet been identified. We have used monoclonal antibodies (mAbs) to define antigenic determinants on several populations of DRG neurons projecting to the superficial dorsal horn of the rat spinal cord.

Three mAbs recognize defined carbohydrate epitopes associated with lacto- and globo- series glycolipids that constitute stage specific embryonic antigens (SSEA's) 1,3 and 4 (Solter and Knowles, Curr.Top.Dev.Biol.13,139,1979). SSEA-3 and SSEA-4 are present in the cytoplasm of about 10% of adult DRG neurons. These neurons project to laminae I and III of the dorsal horn and are distinct from those that contain substance P (SP) and somatostatin (SRIF). SSEA-1 is present in a small percentage of adult DRG neurons. SSEA's are present on the surface of DRG neurons maintained in dissociated cell culture: 6% are $SSEA\text{-}1^{+}$, 6% are $SSEA\text{-}3^{+}$ and 10% are $SSEA\text{-}4^{+}$.

MAbs LD2 and KH10 identify different epitopes expressed coincidently in 25% of small DRG neurons that project to lamina II of the dorsal horn. All SRIF- but less than 1% of SP-immunoreactive DRG neurons express the LD2 and KH10 epitopes. MAb LA4 labels a distinct population of small DRG neurons that also projects to lamina II. Antigens recognized by mAbs LD2, KH10 and LA4 (SNAC antigens) are expressed on the surface of 10-20% of DRG neurons in culture. Preliminary biochemical studies suggest that the SNAC antigens may also be glycolipids.

Other classes of primary sensory neurons are $SSEA^{+}$ and $SNAC^{+}$. However, these antigens are not expressed by central neurons. Molecules bearing these antigens may be involved in the development and differentiation of sensory neurons.

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291.13 ATP RELEASE FROM THE DORSAL HORN OF RAT SPINAL CORD. K.Yoshioka* and T.M.Jessell, Dept. of Neurobiology, Harvard Medical School, Boston MA 02115.

ATP produces a selective excitation of a subpopulation of dorsal horn neurons grown in dissociated cell culture (Jahr and Jessell, Nature 304,730,1983). To provide further information on the possible role of ATP as a synaptic transmitter in the dorsal horn we have examined the release of ATP from rat spinal cord.

The release of endogenous ATP was measured from homogenates or from crude synaptosomal preparations of adult rat spinal cord using an on-line luciferin-luciferase assay with a sensitivity of 10 fmol ATP. ATP release from spinal cord synaptosomes could be evoked by veratrine alkaloids (185 µg/ml) and evoked release was completely blocked by TTX (300 nM). To determine the origin of released ATP we prepared, separately, homogenates from the dorsal region of the spinal cord (laminae I-IV), the intermediate region (laminae V,VI,X and the dorsal part of lamina VII) and the ventral region (lamina VIII,IX and the ventral region of lamina VII). Veratrine-evoked release of ATP from the dorsal region (1.06 +/- 0.07 pmol ATP/mg protein, mean +/- SEM ; n=6) was significantly greater than that from the intermediate region (0.28 +/- 0.04 pmol/mg protein) and that from the ventral region (0.08 +/- 0.02 pmol/mg protein). Veratrine elicited little or no release of ATP from homogenates prepared from the dorsal columns and dorsolateral fasciculus (< 0.01 pmol/mg protein).

Complete spinal transection at the mid-thoracic level did not affect the release of ATP from the lumbar spinal cord indicating that descending neuronal projections do not constitute the source of released ATP. We are investigating whether release originates from primary afferent terminals or from dorsal horn interneurons. These results are in agreement with similar observations using potassium-evoked depolarization (T.D.White, personal communication) and demonstrate a preferential release of ATP from the dorsal horn of the spinal cord. ATP-sensitive neurons and ATP release sites, therefore, appear to overlap in the spinal cord.

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291.14 RETROGRADE LABELLING OF GANGLION NEURONS AFTER INJECTION OF TRITIATED AMINO ACIDS IN THE SPINAL CORD OF CATS. P. Barbaresi* and A. Rustioni, Depts. of Anatomy and Physiology, Univ. of North Carolina, School of Med., Chapel Hill, NC 27514

In an attempt to identify spinal ganglion neurons that may use glutamic acid as neurotransmitter, the present experiments are based upon evidence indicating that neurons may selectively uptake at their axon terminals and transport retrogradely the same chemical they use as neurotransmitter (or analogue). ^{3}H-D-Aspartate (^{3}H-D-Asp) was used as a marker since it is taken up by the same affinity mechanism as L-Asp and L-Glu. Furthermore, recent observations are consistent with the hypothesis that labelling after injections of ^{3}H-D-Asp is confined to neurons that use aspartate or glutamate as neurotransmitter(s).

In adult cats 1.5 µl of ^{3}H-D-Asp (500 µCi/µl) were injected in the dorsal horn between C3 and C6. Twenty-four to thirty-six hours after injection, cats were perfused with 5% glutaraldehyde. In all cases, autoradiographic grains accumulate over a fraction of neuronal population in ganglia closely related to the injection. The number and cross-sectional area of labelled and unlabelled cell bodies with a nucleolus in the plane of the section were calculated in three ganglia from two different animals (total neurons sampled: 6557). After injection at C5-C6, mean values of labelled perikarya are 2387 µm^2 (S.D.958) in C5 and 4190 µm^2 (S.D.1244) in C6; after injection at C6-C7 mean value of labelled perikarya is 3292 µm^2 (S.D.879) in C7. Mean values of unlabelled perikarya in the respective ganglia are 1096 µm^2 (S.D.585), 1683 µm^2 (S.D.1107) and 1941 µm^2 (S.D.826). Labelled neurons represent 6.45% in C5, 9.6% in C6, and 11.5% in C7 of the counted population. These results are similar to those previously obtained in rats (Rustioni and Cuénod, 1981). Similar to previous data are also the observations from an additional cat in which injection of 0.8 µl of ^{3}H-GABA (500 µCi/µl) at C4 resulted in labelling of 5.3% of the sampled neuronal population of C4 ganglion: mean values of labelled/unlabelled neurons are 1707 µm^2 (S.D.731)/1085 µm^2 (S.D.496). The latter results raise question about the significance of the selective retrograde labelling reported here. Experiments are now underway to verify, by immunocytochemical techniques, whether glutamergic neurons can be identified in the spinal ganglia of cats and, if so, whether the size of their perikarya is comparable to that observed in the autoradiographic experiments after injection of ^{3}H-D-Asp.

291.15 IMMUNOHISTOCHEMICAL IDENTIFICATION OF LEUCINE ENKEPHALIN IN DORSAL ROOT GANGLION CELLS OF THE RHESUS MONKEY. J.R. Roppolo, I.P. Lowe, and W.C. deGroat. Dept. of Pharmacology and Center for Neuroscience, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

Immunohistochemical studies have identified nerve terminals containing neuropeptides, such as vasoactive intestinal polypeptide (VIP), substance P (SP), somatostatin (SS), and leucine enkephalin (L-ENK) in the superficial laminae of the dorsal horn. It is thought that VIP, SP, and SS arise in part from primary afferents whose cell bodies are in the dorsal root ganglia (DRG) and that L-ENK terminals arise from neurons within the central nervous system. However recent studies from this laboratory suggest that in the cat L-ENK may also be contained in DRG cells. The present study was undertaken to determine if a similar localization of L-ENK exists in the rhesus monkey.

Standard immunohistochemical techniques (either PAP or FITC methods) were used to examine L-ENK immunoreactivity (L-ENK-IR) in two rhesus monkeys. Pretreatment with colchicine was not necessary to detect the L-ENK-IR.

L-ENK-IR was identified in many neurons at various spinal cord levels including: cervical (C3 and C5), thoracic (T6 and T9), lumbar (L1 and L7) and sacral (S1 and S2). The L-ENK containing neurons, at all levels of the spinal cord, were small to medium size cells with minor and major axis diameters ranging from 18 x 25 to 45 x 48 µm (mean 24 x 28). These neurons were located throughout the DRG with no particular segregation to a specific part of the ganglia. Fibers exhibiting L-ENK-IR were seen very rarely within the DRG or dorsal roots but were commonly detected in Lissauer's tract at all levels of the spinal cord. Dynorphin A (1-8) and methionine enkephalin were not detected in DRG neurons.

These results raise the possibility that some of the primary afferent projections to the primate spinal cord maybe enkephalinergic and that enkephalins might function as inhibitory modulators in sensory pathways.

291.16 IMMUNOCYTOCHEMISTRY OF SPINAL TRIGEMINAL NUCLEI. L.E. Westrum, R. Costello*, A. Hendrickson and J.Y. Wu, Depts. of Neurological Surgery, Biological Structure and Ophthalmology, Univ. of Washington, Seattle, WA 98195, and Dept. of Physiology, Penn. State Univ., Hershey, PA 17033.

Light (LM) and electron microscopy (EM) are being used to study the distributional patterns and ultrastructural localizations in terminals within spinal trigeminal nuclei of immunoreactivity utilizing selected transmitter-related antibodies. Commercially obtained antisera to m-enkephalin (Enk), substance P (SP), serotonin (5-HT) and antiserum to glutamic acid decarboxylase (GAD) prepared according to the protocol of J. Y. Wu are being used specifically in partes caudalis (PC) and interpolaris (PI). The indirect PAP immunoperoxidase procedure of Sternberger is being used for both LM and EM. In the LM, heavy labelling with SP occurs throughout layers I and II (substantia gelatinosa) of PC but is localized to dorsal and ventral subareas in PI. These are the same subareas receiving dental afferents. Enk has a similar distribution but less intense immunoreactivity. 5-HT has a more diffuse labelling with some densities in the upper laminae and near the tract. GAD reactivity is wide spread but is especially intense in layers I and II, dorsally in PC and ventral near the tract in PI. EM of the SP material shows large varicosing axons and terminals with round synaptic vesicles often with dense core vesicles and asymmetric synaptic sites. Enk-positive profiles are usually smaller with pleomorphic and dense core vesicles and symmetric contacts. 5-HT material shows mainly thin axons and terminals with mostly pleomorphic and occasional dense core vesicles with either symmetric or asymmetric contacts. GAD reactivity in the EM shows small axons and numerous terminals with pleomorphic vesicles and symmetric contacts, sometimes onto other unlabelled round-vesicle terminals. Our results show a clear distributional pattern for each antiserum in separate trigeminal subnuclei with different classes of synapses for each. A unique finding is the selective labelling of the SP and Enk antibodies in subregions of the nuclei that specifically receive dental afferents.

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292.1 POSITRON EMISSION TOMOGRAPHIC (PET) SCANNING IN HUNTINGTON'S DISEASE: STUDIES OF ^{18}F-2-FLUORO-2-DEOXY-D-GLUCOSE [^{18}FDG] UPTAKE IN 12 DRUG-FREE CASES. J.B. Penney, A.B. Young, S. Berent, B.J. Giordani*, D.M. Jewett*, R. Ehrenkaufer* and R. Hichwa*(SPON: M.B. Bromberg). Depts. of Neurology, Psychiatry and Internal Medicine, Univ. of Michigan and VA Medical Center, 1103 E. Huron, Ann Arbor, MI 48104.

Huntington's disease (HD) is an inherited autosomal dominant disorder characterized by onset in midlife of abnormal involuntary movements and progressive dementia. Recent PET scan studies in a mixed group of treated and untreated HD patients (Kuhl et al., Ann.Neurol. 12:425-434, 1982) indicated markedly decreased ^{18}FDG uptake in the caudate nucleus. Decreases were seen even in patients with no atrophy of the caudate nucleus on CT scan.

We have studied 12 drug-free patients with HD. Each patient had a quantitative neurological examination, a videotaped examination, a CT scan and a neuropsychological evaluation prior to PET scanning. Four patients were Stage I according to the Shoulson and Fahn rating scale, 4 patients were Stage II and 4 patients were Stage III. Each patient received 2-10 mCi of ^{18}FDG intravenously and arterial blood samples were collected to calculate local cerebral metabolic rate for glucose (LCMRG) according to standard methods.

Abnormalities of caudate and putamen LCMRG were seen in all patients. In each patient, the changes in LCMRG were significantly greater than the apparent structural changes as seen by CT scan. Cross-sectional plots of metabolic activity at the level of the caudate and putamen were obtained. The ratio of caudate metabolism to cortex (but not the intercaudate spacing at half-maximal caudate activity) correlated with stage of disease and degree of cognitive dysfunction. The ratio of the putamen activity to cortex (but not the interputamen spacing) correlated with the degree of motor dysfunction. Tests of visual-spatial and motor performance were also correlated with the subcortical metabolic changes.

Thus, LCMRG as determined by PET scanning can yield useful information about the anatomical localization of specific motoric and cognitive functions. Possible presymptomatic changes in HD are now being investigated in a series of subjects at risk for HD in whom genetic linkage marker data (Gusella et al., Nature 306:234-238,1983) are also being determined.

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292.2 HUNTINGTON'S DISEASE, L-PYROGLUTAMATE, THYROTROPIN RELEASING HORMONE AND METABOLITES: IS THERE A CAUSAL INTERRELATIONSHIP? G.K. Rieke, M.S. Cannon* and H. Williams*[1]. Dept. of Anatomy, College of Med., Dept. Entomology,[1] College of Agriculture, Texas A&M Univ., College Station, TX 77843.

The neurotoxic hypothesis for Huntington's disease (HD) states that an endogenous compound or group of compounds destroys neurons within the central nervous system. The neuropathology is the basis for the debilitating features of the disease (dementia and movement disorders), but the toxins remain unknown. We have measured by GC-MS plasma levels of L-Pyroglutamic acid (L-PGA) in a small number of patients with HD (n = 6) and found significantly elevated levels of L-PGA. The levels were as high as 21X the mean (X = 300 µMoles/L) found in the age-matched nonchoretic controls. Studies in mice using intrastriatal infusion of L-PGA through implanted cannulae in the caudatoputamen (CPU) produced animals showing progressively more severe movement disorders, including rotation, postural asymmetry of the trunk and head, involuntary movements of the head, trunk and contralateral limbs. The neuropathological changes induced by L-PGA (2.1 µg/0.46 µL/hr to 0.42 µg/0.46 µL/hr, pH 7.1-7.2) included a marked depopulation of neurons in the CPU. The lesion consisted of a central necrotic zone filled with macrophages and a peripheral spongiose zone that contained vacuolated profiles and degenerating neurons. In those animals where L-PGA entered the lateral ventricle, the CPU was shrunken and a spongiose zone extended (600-700 µm) from the ventricular surface into the substance of the CPU. Nemeroff and associates (Sci., 221:972-975, 1983) have reported that the levels of thyrotropin releasing hormone (TRH) in the caudate nucleus from HD patients are significantly elevated as compared to controls. Significantly, L-pyroglutamate is the -N-terminal amine of TRH and is one metabolite of TRH. We are presently evaluating the potential neurotoxic actions of TRH administered through Alzet mini-osmotic pumps coupled to an intra-CPU implanted cannula in mice. Three distinct concentrations of TRH are being administered such that the dose/24 hr ranges from 2 to 10 times the normal amounts of extrahypothalamic TRH in mouse forebrain. If TRH proves to be neurotoxic then possible etiological relationship between abnormal TRH levels, the TRH metabolite L-PGA and Huntington's disease will require further elucidation.

292.3 QUANTITATIVE ANALYSIS OF DENDRITIC ALTERATIONS IN BRAINS OF SCRAPIE INFECTED HAMSTERS. R.N. Hogan*, S.B. Prusiner* (SPON: M.P. Daniels). University of California at San Francisco, San Francisco, CA 94143.

Alteration in the morphology of neuronal dendrites has been shown to be a primary neuropathologic manifestation of numerous mental retardative and dementive diseases. Recently, qualitative changes in dendrites were found in biopsy material from patients with Creutzfeldt-Jakob Disease. In order to ascertain whether the changes noted were valid when statistical and quantitative methods were applied to multiple brain areas, a controlled study of scrapie infected hamsters was performed. Weanling hamsters were inoculated intracerebrally with 10^8ID$_{50}$ units of a hamster adapted isolate of the scrapie agent. Clinically positive animals were sacrificed at 11 weeks after infection. The left brain was processed for standard H & E and for rapid Golgi analysis of dendritic spines. The right brain was processed using the Golgi-Cox technique. Golgi stained layer III pyramidal neurons from motor and visual cortex were found to exhibit two types of changes: (1) Spherical swellings of dendritic stalks ranging from 7 to 25 micra in diameter. The average number of swellings per cell was 18.13 with 9.63 occurring on basal dendrites, 7.10 on oblique and 1.4 on apical dendritic trees. (2) Loss of dendritic spines ($p < .001$) on the apical shaft of both motor and visual pyramidal neurons at distances from 50 to 200 micra from the cell body. Dendritic swellings were also noted on hippocampal and thalamic neurons and were probably related to vacuoles seen in H & E sections. Other studies from our lab have shown that prions causing scrapie polymerize into rods which by electron and polarization microscopy resemble the amyloid of Alzheimer's disease. This, in addition to dendritic swelling and spine loss suggest similar mechanisms might be responsible for the functional deficits in Alzheimers and prion diseases. Supported by NIH Grants AG02132 & NS14069.

292.4 EEG AND EVOKED POTENTIALS IN SCRAPIE, A CNS SLOW VIRUS INFECTION OF SHEEP. G.M. Strain, B.M. Olcott* and W.F. Braun, Jr.*. Vet. Physiol., Pharmacol. and Toxicol., and Vet. Clin. Sci., Louisiana State Univ. Sch. Vet. Med., Baton Rouge, LA 70803-8420.

The subacute spongiform encephalopathies (scrapie, Creutzfeldt-Jakob disease, kuru) are CNS infections caused by slow viruses that produce a spongiform encephalopathy, degeneration of neurons, and proliferation of astrocytes. The EEG of CJD is very characteristic, consisting of high voltage periodic complexes that are bilaterally synchronous and symmetric in all leads, and a cyclic alternating pattern of high voltage slow waves and low voltage fast waves. The EEG in kuru is not remarkable. We recorded the EEG, BAEP and flash VEP in 3 Suffolk ewes with naturally-occurring scrapie and 3 controls.

EEG changes seen in sheep with scrapie included semi-periodic polyphasic high voltage complexes, bilaterally synchronous and symmetric in all channels, and cyclic alternating patterns consisting of a high voltage low frequency phase, followed by a low voltage high frequency phase. The high voltage phase occurred with increased arousal, while the low voltage phase occurred with decreased arousal. Myoclonic jerks were time-locked to the occurrence of EEG complexes in one animal. Photic stimulation appeared to evoke polyspike discharges, and several spontaneous focal seizures were observed. Waveform amplitudes were greatly reduced in the BAEP and flash VEP, although degree of reduction did not always correlate with disease severity. EEG and EP changes were seen in an exposed sheep that had not yet developed clinical signs.

Scrapie recordings resembled those of CJD in many aspects. The major difference was in the occurrence of high voltage complexes: those in scrapie did not exhibit the frequency and regularity required in the diagnosis of CJD. Differences in the EEG of scrapie, CJD and kuru are discussed in light of the pattern of brain lesions seen with the three diseases.

292.5 DEOXYGLUCOSE UPTAKE AND CHOLINE ACETYLTRANSFERASE ACTIVITY IN CEREBRAL CORTEX FOLLOWING LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS. M.V. Lamarca* and H.C. Fibiger. Div. Neurol. Sci., Dept. Psychiat., University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

There is substantial evidence that the cholinergic systems that originate in the basal forebrain and that innervate the telencephalon degenerate in Alzheimer's disease (AD). For example, the number of large neurons in the nucleus Basalis of Meynert is decreased in the brains of patients dying with AD, and decreased cortical and hippocampal choline acetyltransferase (ChAT) activity appears to be a consistent correlate of this condition. Recent studies have indicated that glucose metabolism in the neocortex is also reduced in AD. The present experiments investigate the extent to which these observations are related by examining the effects of lesions of the nucleus basalis magnocellularis (nBM) on cortical glucose utilization in the rat.

The nBM of rats (300g) was unilaterally or bilaterally lesioned by pressure injection of a 1µl solution of ibotenic acid (0.5% w/v in phosphate buffer, pH 7.4). Seven days after the lesions, animals were injected i.p. with (^{3}H) 2-deoxyglucose (2-DG, 200 µCi/kg). Some animals received pentobarbital (40 mg/kg) 30 min. before the 2-DG injection. Rats were decapitated 45 min. after receiving 2-DG and various brain regions were assayed for their content of radioactivity as well as for ChAT and glutamic acid decarboxylase (GAD) activities.

Lesions of the nBM decreased ChAT activity by approximately 50% in the ipsilateral anterior and middle cortical regions. ChAT activity in the posterior cortex was also reduced significantly although to a smaller extent. ChAT activity in other brain regions was not affected by the nBM lesions. GAD activity was unchanged in any of the brain areas examined. No change was found in the accumulation of 2-DG in any region ipsilateral to the lesion. Animals with bilateral lesions of the nBM showed similar decreases in cortical ChAT activity. Again, there was no change in 2-DG accumulation relative to sham operated controls. Pentobarbital treatment reduced 2-DG accumulation by approximately 40% in all brain areas studies and this reduction was not affected by nBM lesion.

The results indicate that a decrease in the cholinergic innervation of the cortex does not influence cortical glucose utilization. It appears unlikely, therefore, that the decrease in cortical glucose utilization in AD is related to degeneration of the nBM-cortical cholinergic projection.

292.6 TOPOGRAPHICAL LOSS OF LOCUS COERULEUS CELLS IN ALZHEIMER'S DISEASE. M.P. Lockhart,* C.J. Gibson and M.J. Ball, Dept. of Pathology, University of Western Ontario, London, Ont., N6A 5C1.

Alzheimer's Disease (AD) is a disabling, dementing disease of the elderly in which there is intellectual impairment (loss of memory, language difficulties, visual-spatial deficits) with relative preservation of sensory and motor functions early on in the disease. Ten to 15% of the population over 65 years suffers from some degree of dementia. Deficits (cell number and NT synthesis) of two brain neurotransmitter (NT) systems are reported in AD -- the cholinergic cells of the basal forebrain complex which project topographically to hippocampus and cortex and the noradrenergic cells of the brainstem nucleus, the locus coeruleus and subcoeruleus (LC-SC). In serial cresyl-violet stained sections throughout the entire length of the LC-SC we have confirmed a loss of nucleolated, pigmented cells compared to age-matched controls (ranging from 30 to 80% of total cells). The most severe losses of NE cells occur in presenile cases of AD (onset prior to 65 years of age). There appears to be a topographical loss of cells from LC-SC, with the greatest cell loss seen in anterior sections. This topography is also reflected in a heterogeneous loss of NE in terminal regions -- with significant loss (50-60%) seen in hippocampus; inferior temporal cortex and frontal cortex (particularly, in presenile cases) and slight (30%), but not significant reductions, in hypothalamus and cerebellum. Pathological diagnosis was morphometrically confirmed by Dr. M.J. Ball, U.W.O. Dementia Study -- (Medical Research Council of Canada (PG21), National Institutes of Health (AGNS 03047), Ontario Mental Health Foundation (858)). This study supported by funding to Dr. C.J. Gibson from the J.P. Bickell Foundation; Ontario Mental Health Foundation and Canadian Geriatrics Research Society.

292.7 CSF ACETYLCHOLINESTERASE ACTIVITY DISTINGUISHES SENILE DEMENTIA OF THE ALZHEIMER TYPE FROM OTHER FORMS OF DEMENTIA AND FROM NORMAL AGE MATCHED CONTROLS. S. Gucker, B.A.‡ M. Folstein, M.D., L. Oshida, B.A‡, J. T. Coyle, M.D., L. Tune, M.D. Johns Hopkins University School of Medicine Department of Psychiatry and Behavioral Sciences, Baltimore, MD 21205, USA.

Because of the recent demonstration of a cholinergic deficit in patients dying with Alzheimer's Disease (AD), several laboratories have attempted to identify cholinergic parameters, particularly acetylcholinesterase (AChE), as markers for the presence and severity of disease. In the current study we have measured acetylcholinesterase activity in CSF samples from 36 patients. This includes 12 patients with Alzheimer's Disease, 12 normal controls, and 12 patients with other dementing illnesses. AChE activity was measured in 47 normal patients whose ages ranged from 20 to 84 to evaluate the affect of age on AChE activity. CSF from patients with SDAT showed significantly lower AChE activity than age matched controls and patients with other dementia syndromes. No significant correlation was found between duration of illness, age, severity of illness (as measured by the Mini-Mental State Examination Score), and CSF AChE activity in Alzheimer's Disease. AChE increased significantly over the age range of 20 to 84. These results suggest that AChE activity may prove to be a useful diagnostic test to confirm the clinical diagnosis of moderate to severe AD.

292.8 CHOLINERGIC AND NORADRENERGIC SYSTEMS IN AGING, ALZHEIMER'S DISEASE AND DOWN'S SYNDROME. P.L.McGeer, E.G.McGeer, J. Suzuki*and M. Norman*. Kinsmen Laboratory of Neurological Research, Dept of Psychiatry, Univ. British Columbia, Vancouver, B. C., Canada, V6T 1W5.

Using a specific antibody for human choline acetyltransferase (ChAT), it has been shown that the giant neurons of the substantia innominata (SI or nucleus basalis of Meynert) are exclusively cholinergic. Counts of these neurons, which are the principle source of cholinergic innervation of the cortex, indicate that the number drops from 400,000-500,000 in young controls to about 140,000 in the elderly. Cell counts in cases of senile dementia of the Alzheimer type (SDAT) range from 45,000 to 100,000 cells, suggesting that 100,000-140,000 is the threshold for decompensation. In our hands, there is a parallel loss of cortical ChAT with "normal" aging, with levels in SDAT being 20-50% of age-matched controls; there is an excellent correlation between SI cell counts and the cortical ChAT levels.

Counts of pigmented, noradrenergic (NA) neurons in the locus coeruleus (LC) in controls indicate a loss with age as reported by Brody (Neurobiol. Aging 3:177,1976). Our data suggest about 30,000 cells per LC at birth with a 50% loss by about age 80. SD cases had from 25-100% of control, with the larger losses appearing in the younger cases; this confirms literature reports (Bondereff et al. Neurology 32: 167, 1982; Mann et al. Neurobiol. Aging 2:57, 1981).

The cause of mental deficiency in Down's syndrome is unknown. Cases dying at >40 years show the characteristic histopathology of SDAT and have been reported to show more severe losses of ChAT and NA (Yates et al. Lancet 1980ii, 979; 1981ii, 39). We have recently examined the brain of a male with Down's syndrome dying at 5.5 months and compared the histopathology and biochemistry with that found in our SDAT cases. This infant had 211,250 cholinergic cells in the SI and 27,600 NA cells in the LC. These are both higher than found in SDAT cases. The NA cells are in the normal range. The number of cholinergic cells is about 50% of a normal complement at birth but is still above the level for decompensation. These data suggest that the mental deficiency in Down's syndrome cannot be explained entirely on the basis of a congenital lack of SI and/or LC cells.

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292.9 TEMPORAL VARIABILITY OF SINGLE-TRIAL BRAIN STEM EVOKED RESPONSES IN DOWN SYNDROME INDIVIDUALS. G. C. Galbraith. NRRC-UCLA Research Group, Pomona, CA 91766.

Studies of long-latency sensory evoked potentials in Down syndrome (DS) individuals have reported delayed latencies and larger amplitudes than persons with other forms of mental retardation, or nonretarded controls. By contrast, studies of the short-latency auditory brain stem evoked response (ABR) have shown an opposite pattern, i.e., shorter latencies and smaller amplitudes in DS individuals. DS individuals also show a pattern of interwave conduction times in which certain segments of the conduction pathway are significantly faster, while others are significantly slower.

Components of the ABR are thought to depend upon 'synaptically secure' neurons which discharge with brief and stable latencies. However, the observation of altered latencies, amplitudes, and interwave conduction times suggests that synaptic timing may be disturbed in DS individuals. Thus, the reduction in ABR amplitudes could be due to temporal variability (time 'jitter') of neural events underlying the ABR.

To test the hypothesis of increased temporal variability in DS individuals, we performed a latency compensation analysis (LCA) of single-trial recordings of the ABR. In LCA, individual trial samples are cross-correlated with the total ABR waveform, and a revised ABR waveform is computed by removing much of the trial-to-trial temporal variability. The resulting waveform typically shows enhanced component amplitudes, depending upon the degree of temporal variability inherent in the data. It is also possible to directly quantify temporal variability by computing the standard deviation of correlation lag values available from the analysis.

In the study we report here, we compared university undergraduates (N = 9) and DS individuals (N = 9). LCA was applied to ABR wave V, which was well defined for all subjects, using both a 0.1 ms square wave click and a 2K sine pulse.

The most obvious question is whether control and DS groups differ in trial-to-trial variability in the ABR. A repeated measures analysis of variance showed significantly greater variability for the DS group ($F = 8.894$, $df = 1,16$, $p < .01$). This finding is supportive of our hypothesis that central dysfunction in DS is characterized by disturbances in temporal stability of neural events. Additional LCA results will be presented.

292.10 ACUTE INTERMITTENT PORPHYRIA (AIP), POTENTIAL ROLE OF DELTA-AMINOLEVULINIC ACID (ALA) IN CNS MALFUNCTIONS. R.J. Marley* and J.M. Wehner* (SPON: S. Jackson). Instit. for Behavioral Genetics and School of Pharm., Univ. of Colorado, Boulder, CO. 80309.

AIP is an autosomal dominant genetic disorder characterized by a predisposition to acute crises involving PNS and CNS disturbances. The cause of the neurological deficits is not understood, but acute attacks are accompanied by the elevation of ALA levels in serum, urine, and CSF. It has been suggested that many of the CNS manifestations of AIP are due to the interactions between ALA and the GABA system, with ALA acting as a partial agonist. The goals of our study were: (1) to test the hypothesis that ALA may act as a partial GABA agonist, and (2) to develop an animal model for genetic sensitivity to ALA by examining inbred strains of mice.

Three inbred strains of mice (C3H, DBA, and C57Bl) and one outbred line (HS) were tested with two convulsants that interact with the GABA system in the production of seizures. In the first series of tests, inhalation of bis[2,2,2-trifluroethyl] ether (flurothyl, Indoklon) five min after i.p. or ICV injections of ALA at dosages ranging from 0.01 - 1.0 mM was used. Greer and Alpern (Life Sci., 21:385,1977) have demonstrated the involvment of GABA in flurothyl-induced clonic seizures.

Mercaptopropionic acid (MP) causes severe convulsions by reversibly inhibiting glutamate decarboxylase activity (Karlson et al., Biochem. Pharm. 23:3053,1974). In a second series of experiments, i.p. administration of MP was used to measure the effects of ALA on seizures resulting from decreased GABA levels. MP was administered five min after ICV injections of ALA at the same dosages used in the flurothyl experiments.

Using these tests, we observed that: (1) ALA increased the latency to the onset of convulsions in a dose-dependent manner and (2) there are sex and strain differences in ALA sensitivity. Moreover, both i.p. and ICV injections of ALA produced approximately equivalent increases in latency to clonus on the flurothyl test. We conclude that ALA may be altering GABAergic function in the CNS.

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292.11 LOSS OF NEUROFILAMENT AND FOUR OTHER PROTEINS SENSITIVE TO CALCIUM-ACTIVATED PROTEOLYSIS FROM THE BRAINS OF THIAMINE DEFICIENT RATS. P. E. Gallant*, H. C. Pant and F. F. Weight (SPON: Ichiji Tasaki). Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Although it is well known that thiamine deficiency leads to neuronal degeneration, the mechanism of this degeneration is not established. To induce thiamine deficiency, male Sprague-Dawley rats were fed a thiamine deficient diet and were injected with 1 mg/kg pyrythiamine (a thiamine antagonist) 5 times per week. Weakness, ataxia, and opisthotonic convulsions commenced in these rats 11 to 18 days after the initial pyrythiamine injection. If the rats were treated with thiamine before they lost their ability to walk, they would survive, although some had residual ataxia. If the rats were not treated before this time, they would soon lose their righting reflex. After losing the righting reflex, they would die within 1 to 4 days even if thiamine was administered. The residual ataxia in recovered animals and their inability to recover after the righting reflex was lost, suggest that permanent neuronal damage occurs in these thiamine deficient animals. To test this possibility brains from opisthotonic animals and animals that had lost the righting reflex were examined morphologically, and their brain proteins were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Macroscopically the brains of opisthotonic animals exhibited small (petechial) hemorrhages. Biochemically, these brains had an accumulation of the polypeptides that comigrate with rat plasma proteins, especially hemoglobin. These brains also demonstrated a small and variable decrease of five polypeptides: a 200 kilodalton (kd) peptide that comigrates with the 200 kd polypeptide present in purified neurofilaments, and four other high molecular weight polypeptides. The brains of the animals that had lost the righting reflex had more extensive and numerous petechial hemorrhages than the opisthotonic animals had, and biochemically the brains of rats that had lost the righting reflex had a greater accumulation of plasma proteins, and a greater loss of the 200 kd polypeptide, as well as the four higher molecular weight polypetides (all greater than 200 kd). Since the neurofilament peptide and the four high molecular weight peptides are preferentially degraded by calcium-activated proteolysis in vitro, the results suggest that neuronal degeneration in thiamine deficient rats may be associated with an elevation of intracellular calcium.

292.12 MITOCHONDRIAL ABNORMALITIES IN FIBROBLAST LINE GM 3093 DEFECTIVE IN OXIDATIVE METABOLISM. M. A. Greenwood*, G. Constantopoulos*, S. H. Sorrell*, W. J. Meyer* and J. A. Barranger*. (SPON: R. Porter), NINCDS, National Institutes of Health, Bethesda, MD. 20205.

Fibroblast line GM 3093 (available from the Human Genetic Mutant Cell Respository, Institute for Medical Research, Camden, NJ) was derived from a now deceased 3 yr old girl with severe diffuse neurologic disease and persistent lactic acidosis. Studies with intact fibroblasts suggested a defect affecting the tricarboxylic acid cycle and cell homogenates had deficient activity of the pyruvate dehydrogenase complex (PDHC), P. J. Blass et al (J. Clin. Invest., 51, 1845-1851, 1972). In order to further localize the defect in this cell line, we measured in cell homogenates, the activities of several mitochondrial and non-mitochondrial enzymes. The activities of the mitochondrial enzymes examined as percent of control values were: pyruvate dehydrogenase complex, 21.4; dihydrolipoamide dehydrogenase, 29.7; α-ketoglutarate dehydrogenase complex, 25; cytochrome C. oxidase, 19; isocitrate dehydrogenase, 46.3; glutamate dehydrogenase, 34.7, suggesting a generalized defect in the mitochondria (Constantopoulos et al, Trans. Am. Soc. Neurochem., 14, 119, 1984).

Electron microscopic examination revealed a paucity of mitochondria averaging 20% of the amount found in the control fibroblast lines of approximately same passage number. The mitochondria were elongated or round in shape and did not appear swollen. Cristae were not aligned in parallel arrays. They appeared vesicular or broken and did not fill the entire mitochondria. Some mitochondria contained electron dense debris and few remnants of cristae. No other nuclear or cytoplasmic abnormality was observed. This is the first report of abnormal mitochondria in fibroblasts from a genetic disease involving oxidative metabolism. The results suggest fibroblast mitochondria can be useful in the study and diagnosis of such disorders.

292.13 INTRACRANIAL ALUMINUM PRODUCES AVOIDANCE LEARNING DEFICIT IN IMMATURE RABBITS. M.H. Lee, A. Rabe, J. Shek, and H. M. Wisniewski*. NY State Office of Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Aluminum-induced neurofibrillary changes (NFC), although ultrastructurally and biochemically different from those found in Alzheimer's disease, are still the best model to study the effect of NFC on neuron function. The immature rabbit may be a particularly useful animal to study the role of aluminum-induced NFC in impairing cognitive function: a single intracranial injection of aluminum produces widespread NFC, but the other neurotoxic effects of aluminum are less severe than in the adult rabbit. We have already demonstrated a spatial learning deficit in the immature rabbit (Exp. Neurol., 1982, 76, 441-446) and now report an avoidance learning deficit similar to that reported for the aluminum-treated adult rabbit (Petit et al., Exp. Neurol., 1980, 67, 152-162). We used the same one-way step-down active avoidance task, but our procedure differed from theirs in several other respects.

On day 15, rabbits were given a 50µl cisterna magna injection of either 1% $AlCl_3$ solution (Al, n=10) or physiological saline (S, n=11). Fourteen days later, the Al animals which had not developed neurological signs and the controls were placed on an elevated platform, a tone was sounded for 10 sec and then followed by a 1.5 mA foot shock. After an escape to the platform below, the shock and tone were terminated. Eventually the rabbits learned to avoid the shock by stepping down in response to the tone alone before the onset of shock. Each rabbit was trained in one day to a criterion of 5 consecutive avoidances. Retention was tested one day and again one week later. The Al rabbits showed a significant learning deficit both in terms of the total number of trials and the number of shocks they received (median trials: S 12, Al 39, $p<.002$; median shocks: S 10, Al 23, $p<.02$). No significant retention deficit was observed either after 1 or 7 days. This lack of retention deficit is at variance with such a deficit reported by Petit et al. for the adult rabbit.

Histological analysis demonstrated widespread NFC in the cerebrum, brain stem, and spinal cord of the immature rabbit. This again confirms that aluminum-induced NFC are associated with learning deficits. However, the extent to which other neurotoxic effects of aluminum contributed to the functional impairment still remains to be determined.

292.14 TRIMETHYLTIN-INDUCED LESIONS IN THE MOUSE BRAIN. D.W. Cockerill*, and L.W. Chang. Dept. of Pathology, Univ. of Ark. for Med. Sci., Little Rock, AR 72205.

Trimethyltin (TMT) compounds are known to be potent neurotoxicants. The acute toxic effects of TMT by a single exposure in mice have been previously studied (Chang, L.W., et al., Environ. Res. 29:435-444, 1982; Chang, L.W., et al., Environ. Res. 29:445-458, 1982; Chang, L.W., et al., Environ. Res. 30:399-411, 1983). The purpose of this experiment was to compare and contrast the neurotoxic effects of three treatment regimens with the same total dosage. Male CD-1 mice were administered TMT-Cl by intraperitoneal injection for a total dosage of 3.0 mg TMT-Cl/kg b.w. One group (A) received 1.0 mg TMT-Cl/kg b.w. on three consecutive days, another group (B) received 1.5 mg TMT-Cl/kg b.w. on two consecutive days and a third group (C) received 3.0 mg TMT-Cl/kg b.w. by a single injection. Control animals received a similar volume of saline. Sacrifice occurred 48 hrs after final treatment by intracardial perfusion (2.5% buffered glutaraldehyde) with further fixation by Bouin's solution (light microscopy samples) or buffered glutaraldehyde (electron microscopy samples). Light microscopic analysis revealed extensive necrosis of the granule cells in the fascia dentata and large brain stem neurons of the mesencephalic trigeminal nucleus (MTN) in Group C with slight effects on the Ammon's horn pyramidal neurons. Moderate lesions were noted in the fascia dentata and MTN of Group B, with no remarkable changes noted in the Ammon's horn pyramidal neurons of this group. No noticable changes were observed in Group A brains. Electron microscopy of the hippocampus revealed significant changes in each treatment group. Necrosis and degenerate cells were observed in the fascia dentata of Group C brains with lysosomes and autophagic vacuoles being noted in the Ammon's horn pyramidal neurons. Group B animals displayed lysosomes, autophagic vacuoles, and edema in both the fascia dentata and Ammon's horn pyramidal neurons. Lysosomes and autophagic vacuoles were observed in the fascia dentata of Group A brains, with edema being noted in the Ammon's horn pyramidal neurons of this group. Edema and segregation of synaptic vesicles away from the synaptic cleft in synapses (presumably basket cell synapses) were observed in Ammon's horn in Group A brains. These findings are suggestive of a graded pathologic response to treatment regimens utilized, and are related to a hypothesis of hyperstimulatory injury due to TMT toxicity (Chang, L.W. Neuropathology of trimethyltin: a proposed pathogenetic mechanism. Fund. Appl. Toxicol., in press, 1984.)

292.15 EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS CAN BE INHIBITED BY THE DRUG NALTREXONE E. P. Schoener, I. N. Montgomery and H. C. Rauch. Depts. of Pharmacology and Immunology/Microbiology. Wayne State University School of Medicine, Detroit, MI.

Experimental allergic encephalomyelitis (EAE) is a T-lymphocyte mediated autoimmune primary demyelinating disease. It is induced in guinea pigs by an intradermal inoculation of the encephalitogen emulsified in complete Freund's adjuvant (CFA). The encephalitogen can be allogeneic or syngeneic central nervous system (CNS) tissue or myelin basic protein (MBP) isolated from the CNS tissue. Interestingly, another constituent of CNS tissue, galactocerebroside, is immunogenic and can induce an antibody-mediated demyelination, but not EAE. (Saida, T., et al., Ann. Neurol., 9:87, 1981)

Cerebroside, a major component of myelin, is known to bind opiate ligands. We therefore asked the question whether the opiate receptor antagonist naltrexone (NTX) might stabilize and protect myelin from the immunopathologic processes such as those observed in EAE.

Control guinea pigs inoculated with CNS tissue emulsified in CFA developed EAE in from 12-20 days post-challenge. However, when guinea pigs are treated with NTX (20 mg/kg, daily, subcutaneous) prior to and after challenge, they did not exhibit clinical signs of EAE to the same extent as did the controls. CNS pathology was present in these animals, but reduced slightly in severity from the controls.

Our data suggest that NTX treatment can suppress the development of EAE in guinea pigs if the drug is given within 3 days of challenge. However, the CNS pathology remained. This clinical suppression in the presence of residual inflammatory lesions is not unlike that reproted with antigen-induced immunosuppression of EAE by MBP treatment following challenge. (Einstein, E.R., et al., Immunochem., 5:567, 1968)

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292.16 **SEQUENTIAL ULTRASTRUCTURAL STUDY OF SPONGIFORM PATHOLOGY IN MOUSE SPINAL CORD INDUCED BY NEUROTROPIC RETROVIRUS.** J.E. Smith and B.R. Brooks, Neurology Dept., Univ. Wisconsin Med. Sch., Madison, WI 53792, and Neurology Svc., Wm. S. Middleton Veterans Administration Hospital, Madison, WI 53705.

Murine neurotropic retrovirus induced spongiform polioencephalomyelopathy is classified as a slow virus disease because of its long incubation period (4-6 months) before the onset of clinical signs and protracted clinical course. This neurological disease occurs naturally in feral mice and has clinical, electrophysiological, pathological and biochemical features similar to motor neuron disease or amyotrophic lateral sclerosis in man. The ultrastructural pathological changes in slowly developing disease were studied sequentially by quantitative techniques during the middle and late portions of the incubation period prior to the onset of clinically evident disease and early in the course of clinically evident hind-limb weakness. Newborn NIH:N mice were inoculated intraperitoneally with 2×10^3 plaque forming units of a molecularly cloned strain (Cas Br) of murine neurotropic retrovirus. Male mice were perfused intracardially with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer at the following stages: 60, 90, 120, and 150 days. Lumbar spinal cord was processed for light and electron microscopy. Age-matched control males were also prepared and examined. Morphometry was accomplished with a computer-linked digitizer (BIOQUANT).

Spongiform vacuolization was seen as early as 60 days. It reached a peak at 90 days, both in frequency (50% of total number observed) and in size (30 µm maximum vacuole diameter). More than 50% of spongiform vacuoles occurred consistently at the gray-white matter interface of the ventral and lateral anterior horn (lamina IX), while 30% were interspersed in white matter and 15% in gray matter. The vacuoles at the gray-white border are almost exclusively swollen axons; they are myelin sheathed and almost devoid of organelles. Because of their location in transverse sections and their intermittence along the cord, we hypothesize that they may belong to propriospinal neurons. The spongiform vacuoles in the gray matter are primarily astrocytic ghosts, swollen to an extreme size. These are also largely devoid of organelles except for normal-appearing mitochondria. There are no quantitative changes in the sizes of synapses and small dendrites in the neuropil surrounding anterior horn cells, even at 150 days when evidence of neuronal death is present.

(Supported by a grant from the National ALS Foundation.)

292.17 TUMORAL AND PERITUMORAL CEREBRAL BLOOD VESSELS IN A RAT GLIOMA MODEL. C.L. Farrell*, P.A. Stewart and R.F. Del Maestro*. Brain Research Laboratory, Victoria Hospital, University of Western Ontario, London, Ontario.

The structural and permeability characteristics of blood vessels associated with cerebral tumors change as the tumor grows. These changes give rise to cerebral edema, a major determinant of mortality and morbidity in such patients. They also determine the passage of chemotherapeutic agents from the blood into various areas of the tumor. It is uncertain whether only vessels within the tumor become permeable or whether vessels in peritumoral brain also leak.

We have developed an animal model for primary gliomas in which C6 glioma cells are grown as spheroids and implanted into the cerebral hemisphere of Sprague-Dawley rat hosts. The advantage of this model is that the tumor-brain interface remains distinct, permitting an accurate identification of tumor vs peritumoral brain. In addition, in the 0.5-1 mm zone surrounding the tumor-brain interface, tumor cells could be seen in association with blood vessels, presumably migrating along the perivascular spaces. We have identified two distinctive vascular phenomena in these tumors: a) proliferating blood vessels invade the tumor and differentiate into typical, permeable tumor vessels, and b) tumor cells migrating along established blood vessels alter their endothelial structure such that the vessels become more permeable. However, other vessels in pertumoral brain that are not intimately associated with tumor cells appear to be normal.

To evaluate the cerebral edema associated with these tumors we have quantitated Evan's Blue (EB) extravasation after 1 hour of circulation time. We found that tumor vessels allow EB leakage from the time of earliest vessel ingrowth (5 days in vivo), however, measurable amounts of EB were found in peritumoral brain only when the tumors reached a critical size.

These results suggest that peritumoral edema may originate from both vessels within the tumor mass and from vessels peripheral to the tumor.

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292.18 SURFACE MORPHOLOGY OF THE CENTRAL CANAL IN EXPERIMENTAL HYDROCEPHALUS-HYDROMYELIA. A SCANNING ELECTRON MICROSCOPE STUDY IN THE CAT. K. Rascher*, K. Booz*, E. Donauer* and A.C. Nacimiento; Dept. of Anatomy and Neurosurgery Research Laboratory, Saarland University School of Medicine, 6650 Homburg FRG.

A hydrocephalic-hydromyelitic condition was induced in adult cats by closing the lateral aperatures with intracisternal injections of Kaolin. After initial symptoms characteristic of increased intracranial pressure, which lasted about 10-14 d, the animals recovered. From this time onward ventriculography revealed a distended central canal and cavities communicating with it. These were almost always directed dorso-ventrally through the grey matter into the dorsal columns. The surface ultrastructure of the central canal in normal cats and in animals which had been hydromyelitic for up to two years was examined in the scanning electron microscope. In normal cats the ependyma of the canal is densely ciliated except for narrow zones along the ventral and dorsal midlines. In these areas the cell apical poles are exposed, revealing countless microvilli. In the treated animals the ependymal cells were extremely flattened and had lost most of their surface profiles such as cilia and microvilli. The apical surfaces were frequently smooth except for a fringe of microvilli at their borders. Large numbers of polymorphous supraependymal cells populated the canal and the transitional area between the canal and the cavities. The walls of the cavities were covered with star-shaped cells with broad lamellar extensions. These cells did not resemble the altered ependymal cells of the distended canal. They appear to be astrocytes. In some of the cavities bundles of nerve fibers could be seen which were not covered by the star-shaped cells. Blood vessels within the cavities, however, were covered by them.

Our findings seem to indicate that a) the ependyma and/or subependymal tissue along the ventral aspect of the canal is better able to withstand pressure than that along the dorsal aspect and, b) the ependyma does not proliferate in order to compensate for the increase in surface area.

292.19 ARACHIDONIC ACID AND LIPID METABOLISM FOLLOWING SPINAL CORD TRAUMA. Royal D. Saunders*, Douglas K. Anderson, and Lloyd A. Horrocks. Department of Physiological Chemistry. Ohio State Univ., Columbus, OH 43210

The metabolism of arachidonic acid and membrane lipids was examined in cat spinal cord following compression injury. The exposed spinal cord of anesthetized, laminectomized mongrel cats was compressed with a 170 g weight for 1 min and 5 min. The amounts of prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostacyclin (measured as 6-keto prostaglandin $F_{1\alpha}$), and thromboxane A_2 (measured as thromboxane B_2) were measured after 1 min and 5 min injury, and at 5, 15, and 30 min following 5 min injury. The levels of prostaglandins E_2 and $F_{2\alpha}$ followed similar profiles and showed 2- to 6-fold increases after 5 min injury above the levels in spinal cord tissue from control laminectomized cats. Prostaglandins E_2 and $F_{2\alpha}$ reached a maximum of 20-fold above control. Thromboxane A_2 did not increase until after removal of the compression when it reached a maximum of 12-fold above control. Prostacyclin levels did not increase significantly. The only significant changes seen in the phospholipids of spinal cord were in ethanolamine plasmalogens and phosphatidic acid. Plasmalogen levels were decreased 13% during the first minute of injury and were decreased 22% at 30 min after 5 min of injury. The plasmalogens are the probable source of most of the free arachidonic acid. Phosphatidic acid levels increased 33% during the first minute of injury and were 160% above control at 30 min after 5 min injury. The cholesterol content decreased 17% after 5 min of injury. The synthesis of arachidonic acid metabolites and degradation of membrane lipids may play a role in the sequence of events leading to irreversible autodestruction and paralysis after spinal cord injury.

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292.20 NEUROLOGICAL AND PHYSIOLOGICAL CHARACTERIZATION OF FLUID-PERCUSSION HEAD INJURY IN THE RAT. C.E. Dixon*, J.D. Glisson*, B.G. Lyeth*, R.J. Hamm*, D.P. Becker, and R.L. Hayes. Dept. of Psychology and Div. of Neurosurgery, Medical College of Virginia, Richmond, VA 23298.

Cerebral concussion is a clinical syndrome associated with immediate and transient impairment of brain function in the absence of gross structural damage. Fluid-percussion models (FP) of concussion produce brain injury by injections of fluid volumes of saline epidurally into the cranial cavity. Pressure waves associated with varying magnitudes of injury are quantified in terms of atmosphere (atm). FP produces initial generalized areflexia and subsequent comatose hypotonia as well as anatomical, metabolic, and cerebrovascular changes similar to those seen following human head injury. FP has been applied to rabbits and cats. No studies have characterized FP injury in rats. The purpose of this study was to characterize neurologically and physiologically a FP model using rats to determine if injury to rats produces changes similar to those documented for FP in other species. Rats were prepared prior to injury by chronically implanting a hollow injury screw over a hole along the sagittal suture. In Experiment 1, rats (n=37) were concussed (.95-4.2 atm) and the duration of suppression of corneal, pinna, flexion, righting, and escape reflexes as well as spontaneous locomotion was scored. Similar to other species, as injury intensity increased, duration of reflex suppression increased; e.g., there was a significant linear relationship between injury level and the duration of suppression of the corneal reflex ($p<0.05$). There was no behavioral evidence of seizure activity. Injury levels greater than 3.6 atm produced irreversible apnea in 50% of the rats (n=14). Examination of perfused brains revealed no gross structural damage at injury levels associated with behavioral suppression (3.0 atm). In Experiment 2, rats (n=5) were paralyzed with curare, intubated endotracheally, and artifically ventilated with 70% N_2O and 30% O_2. Arterial blood pressure, blood gases, and cortical EEG activity were recorded prior to and after injury. FP (2.0-2.8 atm) produced a significant pressure response. EEG records showed no evidence of seizure activity. Blood gases were within normal ranges. Examination of the perfused brains revealed no gross structural damage. All systemic physiological variables responded to FP injury similarly to those reported in other species.

292.21 CEREBROVASCULAR SMOOTH MUSCLE: EFFECTS OF DIMETHYL SULFOXIDE. L.H. Pitts*, A.R. Young*, J. McCulloch and E.T. MacKenzie (SPON: E. Hamel). Dept. of Neurological Surgery, Sch. of Med., Univ. of California; Wellcome Surgical Inst., Univ. of Glasgow and Dept. of Biology, LERS-Synthélabo, Bagneux, 92220-F.

Dimethyl sulfoxide (DMSO) has been reported to lower intracranial hypertension both in experimental and clinical conditions of brain injury, although the mechanism of action of this drug has not yet been clearly defined. One hypothesis for this therapeutic effect is a direct cerebral vasoconstriction (resulting in a reduction in blood volume) that lowers intracranial pressure (ICP). In an attempt to better understand the pharmacology of DMSO, its direct vascular effects were studied on cat middle cerebral arteries *in vitro* and following micro-application to pial arterioles, *in situ*.

DMSO did not constrict isolated cerebral arteries at any of the given concentrations studied (0.1 nM to 0.4 M). In vessels preconstricted by potassium, 5-hydroxytryptamine, prostaglandin $F_{2\alpha}$, or with mechanically raised tone, DMSO (0.1 nM to 10 mM) was without significant effects. However, in concentrations greater than 10 mM, DMSO consistently relaxed the arteries (60-85% of the induced tone) probably due to the hyperosmolarity of the bathing medium.

Likewise micro-application of DMSO (1 µM to 10 mM) around pial arterioles, in chloralose-anaesthetized cats, did not alter arteriolar caliber significantly. Higher concentrations of DMSO (1 g/%) increased arteriolar caliber by 19% ($p<0.05$). Once again, this response appeared to have been mediated as a consequence of solution hypertonicity. DMSO did not modify, *in situ*, cerebrovascular responses to alterations in perivascular potassium ion concentrations.

The findings of this study provide no support for the view that direct cerebral vasoconstriction is responsible for the clinical efficacy of DMSO in lowering ICP. The mechanism of action of DMSO remains to be further investigated, but DMSO would appear to be devoid of potentially deleterious actions on the cerebral vasculature.

292.22 **STEROID AND IBUPROFEN IMPROVEMENT IN NEUROLOGICAL RECOVERY IN HEAD-INJURED MICE.** E.D.Hall. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

The purpose of the presently reported study was to examine in a mouse closed-head injury model the ability of certain agents that have theoretically beneficial actions to promote early neurological recovery. Based upon studies showing that massive doses (15-30 mg/kg i.v.) of methylprednisolone sodium succinate (MP) exert a number of therapeutic effects on the injured cat spinal cord (E.Hall and M.Braughler, Surg. Neurol. 18:320, 1982) that can be considered equally relevant to brain injury, a primary focus was on MP. For comparison, the sodium succinate esters of the glucocorticoids hydrocortisone (HC) and prednisolone (P) were also studied. Furthermore, because of the postulated involvement of certain eicosanoids in the acute pathophysiology of CNS trauma, the cyclooxygenase inhibitor ibuprofen (IBU) and the thromboxane synthetase inhibitor U-63557A were also included.

Unanesthetized Charles River CF-1 mice (18-22 g b.w.) received a 900 g-cm (50 g weight dropped 18 cm) concussive head injury which resulted in immediate loss of consciousness (i.e. loss of righting reflex) or death (approx. 30%). Within 3-5 min after injury, the surviving mice received a 0.1 ml i.v. (tail) injection of either HC, P or MP (15, 30, 60 or 120 mg/kg), IBU (1, 3 or 10 mg/kg), U-63557A (1, 3 or 10 mg/kg) or the aqueous vehicle (V). By 1 hr after injury, all mice regained the righting reflex at which time their neurological status was evaluated by both a "grip test" in which the time that the mice could hang on to a suspended string was measured, and a "string test" in which the performance of the mice while on the string was scored (0-5 points).

Head-injured mice that received a 30 mg/kg MP dose exhibited significantly greater recovery than V-treated ones in terms of both tests. For instance, the mean 1 hr grip test value was increased from 11.1±1.1 (S.E.) secs for V to 17.7±1.9 secs ($p<0.05$) for the 30 mg/kg MP treated. In addition, the number of mice that remained on the string for the full 30 secs was increased from 21.9% to 38.5% ($p<0.05$). Interestingly, MP doses lower and higher than 30 mg/kg were less effective. P was also seen to improve recovery, although a 60 mg/kg dose was optimal. HC was ineffective. IBU in doses of 3 or 10 mg/kg i.v. was found to promote recovery at least as well as the 30 mg/kg MP dose. U-63557A, in contrast, did not improve the 1 hr neurological status.

These results show that MP can indeed enhance recovery from moderate to severe head injury, but that doses way above the conventional range are required. The possible mechanisms of this action will be presented. The role of certain eicosanoids other than thromboxane A_2 in the acute pathophysiology of CNS injury is also suggested by the efficacy of IBU, but not U-63557A.

292.23 METHYLPREDNISOLONE IMPROVES RECOVERY IN A GERBIL STROKE MODEL: INVOLVEMENT OF PROSTACYCLIN. M.J. Lainer*, L.A. Duncan* and J.M. Braughler, CNS Research, The Upjohn Company, Kalamazoo, MI 49001

Male gerbils were lightly anesthetized with methoxyflurane, and the right carotid artery isolated and temporarily occluded for 3 hr using a microvascular clamp. Following recovery from anesthesia, animals were observed continuously for 5 hr (3 hr occlusion:2 hr recirculation) for the following: circling, torso curvature, inability to walk, ptosis, barrel rolling, opisthotonus, seizures, and loss of righting reflex. Animals were given one point each hour for each deficit displayed, except for loss of righting reflex, which received 3 points. Those animals with scores of 2 or more during the first hour of observation were included in the study. Approximately 37% of those gerbils occluded had an average score of 4.5± 0.2 by the third hour. Following occlusion removal at 3 hr, gerbils recovered minimally during the ensuing 2 hr period with scores remaining around 3.2±0.3. A striking improvement to 1.6±0.2 ($p<0.05$) by 2 hr after occlusion occurred in gerbils pretreated wtih 60 mg/kg of methylprednisolone sodium succinate (MPSS) 10 min before occlusion. Lower or higher doses were not as effective. Survival at 1, 2 and 7 days was also improved ($p<0.05$) by the 60 mg/kg dose.

Protective effects of MPSS could be blocked by pretreating animals with ibuprofen (20 mg/kg) 20 min before MPSS. The salutary effects of MPSS in ibuprofen-treated animals could be restored by the subcutaneous administration of 1 mg/kg of 9-beta-methyl-(5Z)-6a-carbaprostaglandin I_2, calcium salt, hydrate (U-61,431F), a stable prostacyclin analog.

In order to assess concurrent biochemical changes, some gerbils were sacrificed by microwave irradiation to the head at 1, 3 or 5 hr. The cortex was removed, powdered under liquid N_2, extracted in HC104 and assayed for lactic acid (LA), pyruvate (P), and adenine neucleotides. LA and the LA/P ratio rose nearly 4- and 5.5-fold, respectively, and ATP, total adenylates and energy charge (EC) fell 4-, 2.5- and 2-fold, respectively, during 3 hr of occlusion. Slight improvement occurred during 2 hr of recirculation; however, only EC returned to precontrol levels. Pretreatment with 60 mg/kg of MPSS only affected EC, which was increased significantly ($p<0.02$) at 3 hrs occlusion.

The results suggest that MPSS can improve recovery and survival in gerbils subjected to 3 hr of unilateral carotid occlusion. Results with ibuprofen and U-61,431F suggest that prostacyclin may be involved in the MPSS effect. Metabolic studies indicate that cortical intermediary metabolism is not markedly improved by MPSS, despite striking salutary effects on neurological recovery and survival.

292.24 NEUROLOGICAL DYSFUNCTION AND BRAIN CATECHOLAMINES LEVELS IN THE GERBIL STROKE MODEL: EFFECT OF NALOXONE, MORPHINE AND CLONIDINE. M. Walley*, D. Boisvert*, G. Baker, T. Nihei*, T. Dewhurst* and R. Walley. Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada, T6G 2G3.

The opiate antagonist naloxone is currently under investigation as a therapeutic agent in acute stroke. We studied the acute effects of unilateral carotid occlusion on neurological status and brain levels of norepinephrine (NE), dopamine (DA) and gamma-aminobutyric acid (GABA) in gerbils. During a 3-hour period following carotid occlusion, animals with neurological deficits, seizures, or no symptoms were treated with naloxone, clonidine or morphine, respectively. In each case, control animals were treated with saline. Drugs or saline were administered under double blind conditions.

All of the clonidine-treated animals showed a reduction in seizure activity following drug treatment, while 80% of the saline-treated animals showed a worsening of seizure symptoms. In the asymptomatic animals morphine produced a significant increase in neurological symptoms. In the animals exhibiting neurological deficits without seizures, naloxone had no significant effects.

Neurochemical analyses of the brains of the saline treated animals showed that NE levels were significantly elevated in the ischemic hemisphere of the animals exhibiting seizures. This effect was significantly reversed by clonidine treatment. In the saline treated animals, DA levels were lower in the ischemic hemisphere in the seizure animals but higher in the ischemic hemisphere in the other groups. Morphine treatment produced a significant reduction in DA levels in the ischemic hemisphere of initially asymptomatic animals. Naloxone treatment had no significant effects on NE or DA levels. The levels of GABA were significantly elevated in the ischemic hemisphere and the degree of elevation was positively related to the severity of the neurological symptoms. However, GABA levels were not affected by drug treatments.

The results of this study do not support a role for naloxone in the treatment of stroke. The elimination of ischemic seizures by clonidine may be of clinical significance and warrants further study.

292.25 NEUROLOGICAL DEFICITS AND EMG CHANGES IN THE HINDLIMBS OF RABBITS FOLLOWING TEMPORARY OCCLUSION OF THE DESCENDING AORTA. S. M. Yuhas* and R. J. Anderson, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

A model of temporary spinal cord ischemia has been described by Zivin et al (Arch Neurol 39: 408,1982) which correlated the degree of neurological deficits with neuropathological damage. The purpose of this study was to determine whether (1) there is a correlation between the neurologic deficits and changes in EMG activity and (2) to determine the timecourse of these changes induced by spinal cord ischemia. Rabbits which had been implanted with chronic EMG electrodes in the vastus lateralis muscle were subjected to occlusion of the descending aorta for 12.5 min. under Rompun/ketamine anesthesia. Four rabbits developed marked neurological signs, 4 showed marginal deficits and 2 did not develop any neurologic impairment. In the affected animals the onset of these signs occurred two days after occlusion and remained constant for 4 weeks. The most prominent signs were loss of reflex responsiveness, increased muscle tone and loss of locomotor activity. Sensation in hindlimb dermatomes was less severely affected with greater paresthesia occurring distally than proximally. In 6 rabbits the neurological effects were bilateral; in 2 the loss was greater on the left side. None of the animals exhibited loss of bladder or bowel function. EMG activity was analyzed using FFT of the full wave rectified data. In all rabbits, regardless of the extent of their neurological deficit, there was a progressive increase in the power of the EMG in the 0-20 Hz band. Since this change does not correlate with the severity of neurological loss, it may reflect accommodation of the animals to the test situation over the course of the study. Conversely, there was a relative increase in the power of the EMG in the 20-40 Hz band only in those animals which were markedly affected neurologically. Therefore, these higher frequency components of the EMG activity may be induced by the lesion and correlate with the observed neurological changes. The results showing a greater loss of motor than sensory activity are consistent with the observations of Zivin (1982) that spinal cord pathology occurred preferentially in the central and ventral gray. The results also suggest that the 20-40 Hz band of the EMG may be a useful and quantitative measure of the severity of the lesion.

292.26 QUANTITATIVE ELECTROPHYSIOLOGIC CHANGES IN THE SLEEP-AWAKE CYCLE OF PERSONS SUFFERING FROM SEVERE HEAD INJURY. L. C. Parsons, R. Chambers* and P. Holley-Wilcox*. University of Virginia School of Nursing and Medicine, Charlottesville, VA 22903.

Investigators continue to search for ways of measuring neuronal recovery in persons with severe head injury (SHI). The purpose of this study was to develop a quantitative method of assessing changes in the sleep-awake cycle of SHI persons. A descriptive correlational research design was used to evaluate 17 SHI persons (14 males and 3 females) and 4 control subjects (2 males and 2 females) ranging in age between 13 and 47 years. Quantitative methods used to measure changes in the sleep-awake cycle included EEG, EOG, EMG, ECG, and respiratory rate. Twenty-six, 24 hour and 6, 12 hour (overnight) sleep studies were performed on the SHI persons while 8 overnight studies were carried out on controls. To compare the sleep-awake patterns of SHI persons and control subjects, criteria previously developed by Parsons, et al., Proc. 5th Eastern Nursing Research Conference, 1982, were used to score electrophysiologic data. These criteria were congruent with those of Rechtschaffen and Kales, A Manual of Standardized Terminology...(1968), where normal electrophysiologic patterns were present. However, due to the diffuse neuronal impairment resulting from SHI, additional descriptive criteria were needed to describe the altered electrophysiologic patterns observed in SHI subjects. For example, depending on the level of consciousness as assessed by the the Glasgow Coma Scale (GCS), light sleep (Stage 2), wakefulness (Stage W) and REM sleep were either absent or profoundly altered in SHI persons. As SHI persons progressed in their recovery and became less comatose, EEG patterns normally expected in stages of lighter sleep and wakefulness began to reappear in predictable sequence. To facilitate comparisons between electrophysiologic findings and behavioral assessments, i.e., the GCS, numerical values were assigned to each stage of sleep in traumatic coma and in control studies. These values, mathematically derived and identified as the Index of Staging (IS), can be used to calculate any desired time interval of sleep. Concurrent validity was established for the expanded criteria through correlations between the IS and the GSC, ($r \geq .77$, $r^2 \geq .60$. These data provide information about neuronal recovery and resequencing of electrical activity following SHI. Research supported by Grant #NU00772 - Division of Nursing.

292.27 EXCITABILITY CHANGES IN DEMYELINATED AXONS DURING REPETITIVE STIMULATION MAY BE MEDIATED BY K+ ACCUMULATION[1]. A. Hernández Cruz* and E.J. Muñoz-Martínez. Dento. Fisiología y Biofísica. Centro de Investigación y de Estudios Avanzados del I.P.N. Apartado Postal 14-740. 07000-México, D.F. MEXICO.

Demyelinated axons fail to transmit trains of impulses. To explain this failure, Rasminsky & Sears (1978) postulated intracellular Na+ accumulation at the nodal region. More recent evidences indicate that demyelination increases the relative importance of K+ currents in mammalian nodes (Chiu & Ritchie, 1981; Sherrat, Bostock & Sears, 1980); this finding raises the possibility that extracellular K+ accumulation may account for the depression and the conduction failure during sustained activity, as it has been shown in other normal axonal systems (Kocsis, Malenka & Waxman, 1983).

In this study, the properties of demyelinated motor axons in cats treated with tullidora (K. humboldtiana) toxins were examined. A single dose of tullidora extracts were orally given to 10 cats. Three to five weeks later hind limb paralysis appeared. Demyelination was found in hind limb nerves of paralysed cats (Muñoz-Martínez & Chávez, 1979). Acute experiments were then conducted under barbiturate anesthesia. Gastrocnemius or tibial nerves were stimulated in the popliteal fossa, and single unitary extracellular potentials were recorded from thin S1 ventral root filaments. A previously desheated portion of the nerves were placed in a perfusion chamber located at 1 cm from the stimulating electrodes. Stimulation at frequencies between 10 and 200 Hz produced minor (<0.05 ms) latency shifts in normal fibres perfused with oxygenated, normal ringer (36°C); slow stimulation (0.1 Hz) under nerve perfusion with solutions containing 12.5 to 15 mM K+ produced a decrease in latency followed by a progressive increase until reversible conduction block was attained. Repetitive stimulation of demyelinated fibres was followed by the same biphasic sequence of excitability changes. Perfusion with 10 mM K+ produced a reversible impairment of the ability to transmit trains of impulses in normal and in demyelinated fibres although the effect was more intense in the demyelinated ones. Perfusion with 0 mM K+ solutions improves the fibre response in all the frequencies examined. The supernormal period was significatively prolonged in demyelinated fibres. Latency shifts and intermittent conduction failure occurs in those fibres showing in addition a prolonged refractory period of transmission. Our results suggest that K+ accumulation may explain these phenomena.

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293.1 INTERCORRELATION MATRIX FOR REGIONAL RATES OF GLUCOSE METABOLISM IN HEALTHY MALES: DIFFERENCES BETWEEN YOUNG AND OLD SUBJECTS. B. Horwitz, R. Duara, and S.I. Rapoport. Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, MD 20205.

We previously showed that intercorrelations between resting regional cerebral rates for glucose (rCMRglc), as determined by positron emission tomography (PET) using [18F]fluoro-deoxyglucose, provide a measure of the functional associativity of different pairs of brain regions (Duara et al., Soc. Neurosci. Abstr. 9: 1171, 1983). Partial correlation coefficients, controlling for whole brain glucose metabolism, were used in the analysis. Having divided the brain into 59 regions, we found, for 40 healthy males (ages 21-83 yrs) in a state of reduced sensory input, that the strongest correlations generally were between bilaterally symmetric brain regions, that there were many statistically significant correlations ($p < 0.01$) among frontal and parietal lobe regions and also among temporal and occipital areas, but that there were few significant correlations between the frontal/parietal and occipital/temporal domains.

We report here on an analysis of the correlation matrix in separate groups (15 individuals each) of young (ages 21-40 yrs) and old (64-83 yrs) healthy males. All were examined by PET in a state of reduced visual and auditory input (Duara et al., Brain 106: 761-775, 1983).

The correlation matrices corresponding to the young and old groups both show statistically significant correlations ($p < 0.05$) between bilaterally homologous regions, as well as numerous significant negative correlations between regions in the frontal lobe and regions in the temporal and occipital lobes. The main differences between the young and old matrices are reductions in the old matrix in the number of significant correlations among frontal lobe regions, (2) among parietal lobe regions, and (3) between frontal and parietal regions.

Although there is no significant decline in resting rCMRglc with age in our subjects (Duara et al., Brain, 1983), our results show a decrease in the number of mutually correlated brain regions in the aged group. If these correlations represent functional connectivity, our results suggest a reduced number of functional interactions in the healthy aged brain.

293.2 LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE FREE MOVING MOUSE DURING TWO STAGES OF THE ACTIVITY-REST CYCLE. T. Jay-Nowaczyk*, M.H. Des Rosiers (SPON : A. McRae-Degueurce). Lab. de Neurophysiologie, Fac. de Med., 69373 Lyon, France and Centre de Recherches en Sciences Neurologiques, Dept. Physiol., Univ. Montréal, Quebec, Canada, H3C 3T8.

Local cerebral glucose utilization (LCGU) was measured in free moving C57BL6 adult mice during two stages of the light dark cycle, on corresponding to a period of rest, and the other to a period of high motor activity. LCGU was obtained using Sokoloff's operational equation from optical densities of 20μ brain sections autoradiographs and from glucose and [^{14}C] desoxyglucose plasma concentrations measured on micro-samples taken in the femoral artery. Arterial pH, pO_2, pCO_2, mean arterial blood pressure and hematocrit were monitored.

In the two groups of animals (day : n = 10 ; night: n = 8), LCGU was heterogeneous in the grey matter, the highest values being found in the auditory regions, the cerebellar and vestibular nuclei. In the white matter, LCGU was low and homogeneous throughout the brain except for the habenulo-peduncular tract. Cerebral glucose utilization was generally found to be lower in drowsy animals during the day than in active animals during the night and the difference was significant (p 0.05) in 8 following structures : the sensori-motor cortex, the auditory cortex, the septal nuclei, the nucleus of the olfactory tract, the basal amygdaloid nucleus, the ventral nucleus of thalamus, the lateral geniculate body, the medial geniculate body. However, the suprachiasmatic nucleus was very active during the day and relatively inactive during the night as reported for rats previously.

This study indicates that the cerebral glucose utilization is minimum during the light phase, which is the normal sleeping time of these animals and suggests we need to be cautious in interpreting results concerning metabolic changes in nocturnal rodents.

293.3 EVALUATION OF A CRANIAL WINDOW PROCEDURE FOR STUDYING CEREBRAL CORTICAL GLUCOSE METABOLISM. D. Pelligrino*, D. Miletich*, R. Albrecht*(SPON: D. Speck). Michael Reese Hosp., Chgo., IL 60616.

Various problems are associated with evaluating the effects of test agents on brain glucose metabolism (CMRgl). Among these are anesthetic effects, poor penetration of test substances into the brain, and difficulties related to CMRgl measurement methods. We evaluated a method for studying the effects of test agents on cortical CMRgl in the awake goat. The method uses 2 chronically implanted cranial windows, one over the parietal cortex of each hemisphere (dura excised). Bilateral superfusions (using mock CSF, CO_2-gassed and warmed to 39°C) are carried out (@ 2-3 ml/min) with one side serving as control and the test agent being delivered in the superfusate on the opposite side. CMRgl changes (based on an internal control) are estimated using a sequential double-label 2-deoxyglucose (2-DG) technique (Altenau, L. Brain Res 153:375, 1978). [^{3}H]2-DG is given iv 1h prior to start of perfusion. [^{14}C]2-DG is given during a 2-2.5h perfusion, 45 min prior to sacrifice. Thus, a comparison of $^{14}C/^3H$ ratios (test vs. control side) in the gray matter tissue exposed to the perfusate should allow for the determination of CMRgl changes. In one series of goats we established that > 1.5h superfusion of labelled test substances leads to changes only in the 1-1.5mm of cortical gray tissue exposed to test perfusate but no changes in any other cortical region (or in blood). In another series we measured (via serial biopsy) a 13%/h rate of decline in parietal gray ^{3}H activity (after the 1h peak level) which was identical when comparing right and left sides and unaffected by Nembutal given at 1-2h after injection. This suggests a similar and constant phosphatase activity on both sides. We tested the effects of norepinepherine (NE) bicuculline (B) insulin (I) and temperature (T) on cortical CMRgl using the above methods. The following CMRgl changes were found: NE(+30%), B(+80%), I(+15%), and T(5-6%/deg Δ T). The values for NE, B and T compare favorably to other reports. The above results show the viability of the described procedure.

293.4 REGIONAL BRAIN GLUCOSE AND ENERGY METABOLISM IN NORMOCAPNIC AND HYPERCAPNIC RATS DURING BRIEF SEIZURE ACTIVITY. A.L.Miller, L.J.Stone,* J.Kwan*. Dept. of Psychiatry, Univ.of Texas Health. Sci. Ctr., San Antonio, TX 78284

Several studies of brain glucose and energy metabolism during prolonged (≥ 20 min) seizures have shown marked differences between regions. An implication of this work has been that the areas most susceptible to damage during status epilepticus are those which are the most stimulated. Comparable studies of brief seizures, attempting to relate metabolic changes to post-ictal changes in function, have been hampered by methodologic problems.

We used focussed microwave irradiation at 915 MHz (25 kW maximum output(Medina, M.A., et al. in Cerebral Metabolism and Neural Function. Williams and Wilkins, 1980) to stop rat brain metabolism in 0.7 s. Rats were anesthetized with methohexital, paralyzed with succinylcholine, ventilated on a respirator, and protected from seizure-induced cardiac slowing or arrest with methscopolamine. Ventilating gases were 97% O_2:3% CO_2 (normocapnia) or 90% O_2:10% CO_2 (hypercapnia). Treated rats were given pentylenetetrazol (PTZ) 100 mg/kg IV 30 s prior to sacrifice. Rates of glucose utilization were determined with [6-^{14}C]glucose and ^{3}H-fluorodeoxyglucose as tracers using a method previously described (Miller, A.L., et al. J. Neurochem. 38:916, 1982). EEG-monitored seizure activity became evident about 10 s after PTZ injection and was continuous until the time of sacrifice.

The largest percent increases in glucose use were in hippocampus (400%), while the most rapid rates were in cortex (3.6 and 1.7 μmol/g/min in normo- and hypercapnia, respectively). Increases in rates of glucose use were much less in brainstem and cerebellum (75 and 115%, respectively). Slight (< 10%) decreases in ATP levels were found in the most activated regions. Creatine phosphate levels fell, concurrent with the development of a lactic acidosis which was greatest in the regions with largest increases in glucose use. In all regions, hypercapnia had three effects compared to normocapnia: (1) baseline rates of glucose use were decreased, (2) PTZ-stimulated rates of glucose use were halved, and (3) accumulation of lactic acid was lessened.

These results illustrate the inhomogeneity of the effects of brief seizure activity on brain glucose metabolism. The most stimulated regions (cortex, hippocampus, and thalamus) control functions, such as memory and cognition, which are most affected by spontaneous or therapeutic seizures. The least stimulated regions (brainstem and cerebellum) control functions which are much less affected. Thus, degree of ictal metabolic disruption correlates with post-ictal dysfunction. Supported by the Morrison Trust, San Antonio, TX.

293.5 LOCAL GLUCOSE METABOLISM OF THE CEREBRAL CORTEX IN A PRIMATE MODEL OF PARKINSON'S DISEASE. R. J. Schwartzman and G. M. Alexander. Dept. of Neurology, Jefferson Medical College, Philadelphia, PA 19107.

Nine monkeys (Macaca fascicularis) were used in this study. Five animals served as controls. Four animals were rendered Parkinsonian by the serial injection of 0.5 mg/kg of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (NMPTP) (Burns et al, 1983) Three animals were injected daily over a 4 day period, one animal was injected weekly over a 4 week period. The animals received a daily neurological examination. Prior to injection of deoxyglucose the animals revealed a flexed posture of trunk and extremities, bradykinesia, increased tone without cogwheel rigidity, loss of vestibular righting reflexes, decreased vocalization and swallowing, failure of upgaze, and abnormal pursuit eye movement. Reflexes were hyperactive. The local cerebral metabolic rate for glucose (lCMRglu) was measured quantitataively, 3 days after the last NMPTP injection, utilizing the method of Sokoloff et al (1977). The lCMRglu was evaluated in selected areas of the sensory, motor, visual, auditory, and cerebellar cortex. The following areas of the cerebral cortex demonstrated a significant ($P<0.05$) decrease in local glucose metabolism: motor cortex area 4, sensory cortex areas 1, 2, 3, areas 17 and 18 of the visual cortex and the primary auditory cortex. The cerebellar cortex demonstrated no change in lCMRglu. The primary motor and sensory cortex were analyzed by layer. In the sensory cortex the greatest decrease in lCMRglu was seen in layers III, IV and V. Area 3a and 3b demonstrated the greatest decrease in metabolism. In the primary motor cortex (4), layer V demonstrated the greatest decrease in lCMRglu. This decrease in functional activity of the cerebral cortex corroborates recent regional blood studies in Parkinsonian patients. It may also underlie the striking decrease in proprioceptive and touch placing responses demonstrated in these animals.

293.6 DOUBLE-LABEL 2-DG TECHNIQUE YIELDS DOUBLE DISSOCIATION OF FUNCTIONAL STATES WITH AN IMAGE DIFFERENCING METHOD. H.R. Friedman, C.J. Bruce and P.S. Goldman-Rakic. Sec. Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

The 2-deoxyglucose (2-DG) method is a technique for mapping metabolic activity in the central nervous system. Double-label versions of this technique, in which two experimental manipulations are directly compared within a single subject, may greatly increase the power of the 2-DG method. We report here a quantitative analysis of a double-label approach using a protocol similar to one described by Livingstone & Hubel (Nature, 291:554, 1981). In our study, ^{3}H and ^{14}C labeled 2-DG were sequentially injected in the same animal while activating first one and then the other hemisphere. Following the injection of the first label, rats received unilateral stimulation consisting of monocular stroboscopic flicker, monaural white noise, tactile stimulation of one side, and/or focal electrical stimulation of cerebral cortex. The laterality of the stimulation was reversed before injecting the second label. Each stimulation epoch lasted 30 minutes. The [^{14}C]2-DG dose was 25uCi/kg; the [^{3}H]2-DG dose was 100 times larger to exploit the differential sensitivity of X-ray and LKB Ultrofilm to these two labels. Control rats were given [^{14}C] or [^{3}H]2-DG at these doses. The same brain sections (20μ), with sets of ^{14}C and ^{3}H standards, were first exposed on LKB Ultrofilm for 10-15 days and then exposed on X-ray film for 30-45 days with a mylar sheet interposed to block ^{3}H radiation.

Our analysis exposed a problem with strict reliance on the two types of film images. Although the X-ray film images reflected, nearly exclusively, the ^{14}C label in the brain, images on the LKB film reflected exposure to both ^{14}C and ^{3}H radiation. Indeed, nearly 50% of the LKB images were due to ^{14}C. Thus, even though the ^{3}H dose was 100 times greater, LKB images were significantly contaminated by the more penetrating β-emissions of ^{14}C and were misrepresentations of [^{3}H]2-DG metabolism. However, a veridical image of ^{3}H label was obtained using a computer algorithm that removes ^{14}C activity, as determined from the X-ray image of the same section, from the LKB image. Using this image differencing method, we identified brain structures with greater levels of ^{14}C in one hemisphere and greater ^{3}H in the other. These double dissociations of label agreed with the laterality of stimulation given for each label, and hence quantitatively validate the 2-DG double-labeling strategy. Supported by NIH and NIMH.

293.7 SEQUENTIAL DOUBLE LABEL FDG AUTORADIOGRAPHY OF RAT BRAIN. J.L. Olds*, K.A. Frey, R.L. Ehrenkaufer*, J. Patoki* and B.W. Agranoff. Neuroscience Laboratory and Cyclotron/PET Facility, University of Michigan, Ann Arbor, MI 48109.

We have previously demonstrated in experiments with ^{14}C- and ^{3}H-labeled deoxyglucose (DG) that the injection of one isotopically labeled form of DG into an experimental animal under control conditions followed 30-60 min later by the injection of the second isotopic form while the animal is in a different physiologic or behavioral state leads to a doubly labeled brain, in which regional $^{14}C/^{3}H$ ratios in tissue punches will reflect a differential metabolic response to the experimental variable (Brain Res. 153:375, 1978). We report here the use of ^{14}C- and ^{18}F-labeled fluorodeoxyglucoses for the purpose of autoradiographic imaging of both conditions. In initial validation studies, rats were simultaneously injected with 100 μCi/kg of ^{14}C-FDG and 25 mCi/kg of the positron emitter, ^{18}F-FDG ($t_{1/2}$ = 110 min). Arterial plasma was analyzed for ^{14}C, ^{18}F and glucose. At the end of the incorporation period, brains were quickly frozen and sectioned at 20 μm. A 12 h autoradiographic exposure on Kodak SB-5 X-ray film was initiated within 4 h of FDG injection. Three days later, a 1 wk exposure for ^{14}C was started. Computer assisted densitometry, used to determine tissue isotope concentrations, revealed a significant ^{14}C contribution to the ^{18}F autoradiogram, despite the initial 250-fold $^{18}F/^{14}C$ ratio. Aluminum foil (50 μm) interposed between the tissue and film during the ^{18}F exposure reduced the ^{14}C contribution by 90% in subsequent studies. We used the $^{14}C/^{18}F$ method to demonstrate the barbiturate effect on rCMRglc in rats with striatal lesions (Science 219:879, 1983). Rats were lesioned by ibotenic acid injection into the right corpus striatum and were injected 1 wk later with ^{14}C-FDG. They were injected 30 min later with pentobarbital and ^{18}F-FDG. Following a second 30 min incorporation, brains were removed, sectioned, and both immediate and delayed autoradiograms were prepared as described above. The lesioned striatum was found to be selectively refractory to the depressing action of anesthesia on brain metabolism as anticipated, thus demonstrating the efficacy of the autoradiographic sequential double label approach as a quantitative method. (Supported by NIH grant NS 15655.)

293.8 HIGH RESOLUTION DEOXYGLUCOSE AUTORADIOGRAPHY. W. Elsen*, R. Ammelburg* and H. Flohr* (SPON: D.W. Sirkin). Dept. of Neurobiology, University of Bremen, NW 2, 2800 Bremen 33, FRG.

The resolution of the original autoradiographic deoxyglucose technique as proposed by Sokoloff and co-workers is limited to approximately 100-200 μm which does not allow analysis of the cellular or subcellular localization of the tracer. This limited resolution is not essentially due to the use of a diffusible indicator, but to certain details of the procedure, in particular: (1) the tissue drying technique, which causes dislocation of the tracer and considerable damage to the tissue; (2) the freeze sectioning technique which limits slice thickness; (3) the use of contact autoradiography employing high speed material which provides a relatively poor resolution and (in comparison to the dipping technique) no exact relation between histological structures and blackening effects in the separated autoradiograph; (4) the use of ^{14}C as a tracer.

By modifying these steps an improved technique was developed. (^{3}H)-2-deoxyglucose labelled brains were deep frozen, freeze dried at -80°C and $5x10^{-5}$ mbar, and embedded in a water insoluble plastic medium. 2-4 μm sections were cut, covered with emulsion (Ilford K5) using the dipping or the loop technique and exposed for 200-400 days. With this approach the localization of 2-DG in single neurons and subcellular compartments was possible with a resolution of approximately 1 μm. There is no noticeable loss or diffusion of the radioactive tracer in the tissue.

Brain areas that appear homogenously labelled with the low resolution technique can be further resolved into highly structured and variable activity patterns, which closely correlate with characteristic functional states.

293.9 A COMPARISON OF THE EFFECTS OF METHYLPHENIDATE AND AMPHETAMINE ON LOCAL CEREBRAL METABOLIC ACTIVITY. G. Lucignani*, B. Saunders* and L.J. Porrino (SPON: C. Kennedy). Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20205.

The behavioral effects of methylphenidate (MP) are similar to those of amphetamine (AMPH). At low doses both enhance locomotor activity, whereas at higher doses both elicit stereotypic behaviors. The mechanism of action of MP and AMPH, however, are distinguishable on the basis of reserpine pretreatment, which does not inhibit AMPH elicited behaviors and dopamine release, whereas it blocks MP elicited behaviors and dopamine release in vivo (Scheel-Kruger, Eur. J. Pharmacol. 14:47-59, 1971; Chieuh and Moore, J. Pharmacol. Exp. Ther. 193:559-563, 1975). The purpose of this study was to determine whether the reported differences in biochemical action, between MP and AMPH, would result in different patterns of metabolic activity. The quantitative autoradiographic 2-[^{14}C]deoxyglucose method J. Neurochem., 28:897-916, 1977) was used to define the pattern of brain energy metabolism which results from the acute administration of MP and to compare it with that following AMPH administration (Porrino et al., Brain Res., in press). Rates of local cerebral glucose utilization (LCGU) were measured according to the standard protocol in four groups of male adult Sprague-Dawley rats, in which 2.5, 5.0, 15.0 mg/kg MP or vehicle alone, was administered i.v., 15 minutes prior to the initiation of the experimental procedure for measurement of LCGU. MP administration resulted in discrete changes in LCGU at all doses. (1) Significant dose related increases in LCGU were observed within the extrapyramidal dopaminergic system, with the largest increases in the substantia nigra and entopeduncular nucleus; increases were also observed in the subthalamic nucleus. (2) In contrast significant dose related decreases in LCGU were observed in the medial and lateral parts of the lateral habenula. (3) In the anterior cingulate cortex, prefrontal cortex, and nucleus accumbens significant increases in LCGU were observed at the lower doses (2.5 and 5.0 mg/kg) but were not evident at the highest dose (15 mg/kg). Despite differences in their biochemical actions the pattern of LCGU following MP administration is similar to that seen with AMPH, particularly with regard to the nucleus accumbens and the extrapyramidal system. Similar behavioral patterns elicted by both MP and AMPH have been found therefore to correlate with similar specific changes in brain energy metabolism.

293.10 BILATERAL 6-HYDROXYDOPAMINE LESIONS OF THE LOCUS COERULEUS: EFFECTS ON LOCAL CEREBRAL GLUCOSE UTILIZATION. A. Justice*, S. M. Feldman, L. L. Brown. Department of Psychology, New York University, New York, NY 10003.

A number of studies have examined glucose utilization in brains with locus coeruleus (LC) lesions. However, these experiments have either used only unilateral lesions (there are significant contralateral as well as ipsilateral projections from the LC), used electrolytic lesions (which cause nonspecific damage), or measured only relative glucose utilization (e.g., comparisons based on gray matter to white matter ratios). Under these circumstances, interpretation of results can be complicated.

In the present experiment, the LC of 5 rats was destroyed bilaterally with local stereotaxic injections of 6-hydroxydopamine, a neurotoxin specific to catecholamine neurons. Seven control animals were injected with vehicle alone into the LC. Lesions were confirmed using histofluorescence microscopy and cell counts. Ten days after surgery the animals were processed for quantitative determination of local cerebral glucose utilization (LCGU) using the 2-deoxyglucose (2DG) method of Sokoloff et al. (1977).

LCGU was determined bilaterally in 109 structures. With Student's t-test only 6 structures were affected at the .05 level of significance or less, all of which were in the direction of lower LCGU in lesioned animals. Structures affected were the nucleus of the diagonal band of Broca (25% lower in lesioned animals than in controls), nucleus accumbens (20%), anterior commissure (19%), flocculus (19%), somatosensory cortex (17%), and genu of the corpus callosum (25%). (Had a non-quantitative analysis been done, comparing all structures to the genu, the results would have indicated statistically significant increases in 26 structures). There was a general trend toward lower LCGU in lesioned animals (95 structures were lower in lesioned animals, 14 were higher). The pattern of changes did not appear to be random: There were greater decreases in neocortex (11% average decrease in 14 different areas), cerebellar cortex (10% in 8 areas), and white matter (16% in 5 areas) than in hypothalamus (3% in 8 areas) and hippocampus (1% in 10 areas).

293.11 DEVELOPMENTAL CHANGES IN REGIONAL BRAIN METABOLISM OF THE KITTEN. M. J. George*, T. L. Baker, T. S. Kilduff, H. C. Heller, and W. C. Dement. Depart. Psychiatry and Behavioral Science, Stanford Univ. Sch. of Med., Stanford, CA 94305

Regional brain metabolism was assessed in kittens aged 20, 40, 60 and 80 days using the [^{14}C] 2-deoxyglucose (2DG) technique. Behavioral state was monitored via implanted standard electrodes for recording EEG, EMG, and EOG. Kittens were injected during alert wakefulness with 75 μCi/kg of [^{14}C] 2DG via an indwelling jugular catheter. If polygraphic signs of drowsiness or sleep appeared, the kitten was wakened by tapping on the side of the recording chamber. After 45 minutes the kitten was sacrificed with i.v. sodium pentobarbital and the brain was removed and rapidly frozen. The brain was sectioned at -20°C at 20 μm thickness, dried rapidly on cover slips, and exposed to x-ray film for 14 days. Uptake of [^{14}C] 2DG, which is proportional to brain metabolism, was measured by taking optical density readings of 81 brain structures from the x-ray films. Relative [^{14}C] 2DG uptake (R2DGU) was then calculated by taking the ratio of the optical density of each structure to that of the optic tract or the internal capsule, white matter structures which show low metabolic activity and low [^{14}C] 2DG uptake.

Kittens showed significant increases in R2DGU in many brain structures as a function of increasing age. The most consistent age-related increases in brain metabolism were seen in cortical and limbic structures: basal amygdaloid n., lateral habenula, lateral and medial septal n., n. olfactory tract, caudate n., cingulate cortex, auditory cortex, and somatosensory cortex. In contrast, structures in the brainstem and cerebellum did not show significant increase in R2DGU.

These data indicate that metabolic activity of cortical and limbic structures changes dramatically between 20 and 80 days of age in the kitten. The unchanging R2DGU in brainstem and cerebellum throughout this developmental period suggests that the maturational changes in neural activity and rate of metabolism of these structures occur before 20 days of age.

293.12 HISTOCHEMICAL CHANGES IN CYTOCHROME OXIDASE DURING CNS DEVELOPMENT. G.H. Kageyama* and M. Wong-Riley. (SPON: D.A. Riley) Dept. Anat., Med. Coll. Wis., Milw., WI 53226.

Recent studies have shown that cytochrome oxidase (C.O.) can be used as a functional metabolic marker for mature neurons in the CNS. In order to determine if the same pattern existed in the neonate, or if there was a temporal progression in the development of C.O. activity within neurons, we examined brains of 12 kittens ranging from postnatal day 1 to 62, and compared them with the adult. In several laminated structures, such as the olfactory bulb (OB), dentate gyrus (DG), hippocampus (HIP), prepyriform cortex (PPC) and cerebellum (Cb), several developmental patterns of C.O. staining were observed. (1) A shift from a predominantly somatic localization of darkly reactive mitochondria at birth to mainly a dendritic concentration postnatally. This process appeared to coincide with the maturation of major afferent inputs to the system. Examples of this pattern included principal cells of the OB (mitral and tufted), PPC (pyramidal), DG (granule), HIP (CA1 pyramidal) and Cb (Purkinje). In the OB, a postnatal increase in dendritic reactivity occurred not only at the site of olfactory nerve input (glomeruli), but also within the plexiform layers, where dendrodendritic synapses are formed. (2) Some classes of neurons either retained elevated C.O. reactivity within their somata throughout development (CA3 pyramidal cells & some HIP interneurons), or became darkly reactive during this period (some interneurons in OB & Cb). (3) Other cells did not exhibit elevated C.O. reactivity within their somata during the postnatal period examined (OB periglomerular & granule cells, & Cb granule cells). In addition, we found that in the Cb, the basket axon terminals and mossy terminals became progressively more darkly reactive postnatally. The increase in dendritic reactivity occurred during the first 3-4 weeks for neurons exhibiting either patterns 1, 2 or 3, and preceded decreases in somatic reactivity in neurons having pattern 1. Similar patterns were observed throughout the CNS. The decrease in reactivity of Purkinje cell somata occurred with increases in basket axon reactivity. Thus, during postnatal development, the elevated oxidative energy demands of many neuronal somata may be due largely to such heightened functions as protein synthesis and the growth of neuronal processes, while subsequent increases in dendritic C.O. reactivity may be related to dendritic elaboration and maturation of synaptic activity (e.g. active ion transport). (Supported by NIH NS 18122.)

293.13 CEREBROCORTICAL METABOLITES AND IN VIVO CYTOCHROME c OXIDASE REDOX RELATIONSHIPS IN NORMAL AND FLUOROCARBON - CIRCULATED RATS. Avis L. Sylvia and Claude A. Piantadosi*. Departments of Physiology and Medicine, Duke University Medical Center, Durham, N.C. 27710

Differential visible wavelength reflectance spectrophotometry was used to continuously and concurrently measure in vivo changes in cytochrome c oxidase (cyt.a,a3) reduction-oxidation state, hemoglobin saturation (HbO_2/Hb), and blood volume (rBV) in the parietal cortex of skull intact anesthetized and ventilated normal blood circulated (NBC) and "bloodless" rats. "Bloodless" animals were obtained, after splenectomy, by exchange transfusion using the fluorocarbon emulsion FC-43 (Green Cross Corp.) to hematocrits below 1%. In NBC animals, absorbance measurements were recorded during sequential changes in the fractional content of inspired oxygen and carbon dioxide, i.e. normoxia; hyperoxia; hyperoxic hypercapnia; normoxic recovery; hypoxia; and anoxia. In vivo changes in the redox state of cyt.a,a3 were compared directly with in vitro measured changes in cortical metabolites (CrP; ATP; pyruvate; lactate) obtained by conventional techniques of freezing, extraction, and enzymatic analysis. The metabolite profile revealed that increases in cortical cyt.a,a3 reduction level most significantly paralleled a decline in CrP as opposed to ATP concentration. Concurrent studies were performed on FC-43 rats obligatorially maintained hyperoxic ($100\%O_2$). Animals were exposed to hypoxia by sequential reduction in inspired O_2 tension. Hypoxia produced an immediate increase in the reduction level of cyt.a,a3 while Hb saturation and rBV were not significantly changed owing to blood removal by FC-43 exchange. Inspired O_2 concentration could not be reduced below 70% without a precipitous fall in arterial pressure, loss of EEG activity, and subsequent death even though arterial PO_2 was maintained above 300 mmHg. Cerebrocortical metabolite concentrations of CrP,ATP,pyruvate, and lactate were found to be significantly decreased in "bloodless" hyperoxic animals presumably due to enhancement of the Pasteur effect. Additional metabolite profiles during FC-43 circulation are under investigation. Data from these studies indicate that noninvasive optical in vivo assessement of changes in the redox status of cytochrome c oxidase provides a reliable and early indicator of intracellular oxygen insufficiency when compared to conventional measurements of changes in cerebral cortical metabolites.

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293.14 QUANTIFICATION OF CEREBRAL OXYGENATION BY IN SITU MEASUREMENTS OF REDUCTION/OXIDATION (REDOX) CHANGES IN CYTOCHROME OXIDASE: APPROACHES AND LIMITATIONS. B.L. Brizzee* and N.R. Kreisman, Dept. of Physiology, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Cytochrome oxidase (cyt a,a_3) is the terminal member of the mitochondrial respiratory chain that reacts directly with O_2. Dual wavelength reflectance spectrophotometry has provided a non-invasive measure of relative changes in redox levels of cyt a,a_3 in cerebral cortex (Jöbsis et al J. Appl. Physiol. 43: 858-872, 1977) and simultaneous recording of cerebral PO_2 has demonstrated that there is a close relationship between these two parameters (Kreisman et al Brain Res. 218: 161-174, 1981). Quantification of these redox changes is desirable in order to compare cerebral oxygenation levels within and between animals, with the ultimate goal of assessing whether O_2 supply is sufficient to meet demand. One method to quantify cyt a,a_3 redox changes has been to determine the difference between optical signals measured during 'maximal' oxidation (by respiring the animal with 100% O_2 or high O_2/low CO_2 mixtures) and during 'maximal' reduction (by respiring the animal with 100% N_2). This difference has been termed the "total labile signal" (TLS) and experimental values are expressed as a percent of TLS (Sylvia & Rosenthal Brain Res. 146: 109-122, 1978). In evaluating this method, we have found that the redox values typically used to define TLS are, in fact, not maximal. The 'maximal' oxidation level can be exceeded by both the oxidation associated with seizures and the rebound oxidation after brief N_2 respiration. Hyperbaric O_2 has also produced cyt a,a_3 oxidation in excess of this value (Hempel et al J. Appl. Physiol. 46: 53-60, 1979). In addition, the magnitude of redox changes used in determination of TLS are not always consistent, primarily because of variability in cerebrovascular responses to changes in O_2, CO_2, and blood pressure throughout the course of an experiment. While it is apparent that caution should be applied in the use of TLS, and experimental conditions should be specified carefully, these limitations do not negate the utility of this method. Proposals will be offered for a redefinition of TLS and assessments will be made of alternative methods for standardizing cyt a,a_3 redox changes. (Supported by NIH grant NS 17443).

293.15 THE CARBONIC ANHYDRASE INHIBITOR (ACETAZOLAMIDE) INCREASES CEREBRAL BLOOD FLOW AND OXYGENATION. J.C. LaManna, K.R. Sullivan* and K.A. McCracken*. Dept. Neurology, Univ. Hospitals of Cleveland and Depts. Neurology and Physiology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

Acetazolamide (Az) is now being used to prevent headaches which accompany acute altitude hypoxia in man. In an attempt to understand the mechanisms underlying this effect, we examined blood gas and pH status, cerebral blood flow (CBF), and tissue oxygen tension (PtO2) in awake and anesthetized rats after a single intravenous dose of Az (50mg/kg). Femoral arterial and right atrial cannulae were placed in Wistar rats (200-300 g) under chloral hydrate anesthesia. The rats were placed in restraining plaster casts and allowed to recover from anesthesia. Arterial CO2 increased, and pH decreased, during the first hour after Az and then both gradually recovered during the next 5 hrs. Arterial PO2 increased and remained elevated throughout the 6 hour period of observation. Regional CBF was determined by the [^{14}C]-butanol indicator fractionation technique. CBF increased by about 50% within 15 min after Az, remained elevated for 1 hr, and returned to control within 3-6 hr. In another group of rats, kept under chloral hydrate anesthesia, the blood gas and pH response to Az was similar to that of the non-anesthetized group. In this latter group, PtO2, monitored continuously in the parietal cortex using platinum microelectrodes, increased during the first 15 min and slowly recovered towards baseline for the next hour. Taken together, these results indicate that Az increased CBF and PtO2 probably due to the concomitant elevation of PaCO2. During altitude hypoxia, this would have the effect of preventing hypocarbia (and the resultant drop in CBF and PtO2) during compensatory hyperventilation.

293.16 CORRELATION OF MITOCHONDRIAL OXIDATIVE ACTIVITY, METABOLITES AND ECoG AFTER CEREBRAL ISCHEMIA IN RAT. M.B. Harrison*, R. Busto*, M. Ginsberg*, M. Rosenthal, and T.J. Sick. Dept. of Neurology, Univ. of Miami, Miami, FL 33101

Reperfusion following 10 minutes of global ischemia in rat cerebrum is characterized by mitochondrial hyperoxidation and continued ECoG suppression. Subsequent re-reduction of mitochondrial components coincides with ECoG recovery. To interpret this period, ischemia was produced in anesthetized rats by cauterization of vertebral arteries and reversible carotid artery ligation. NAD and cytochrome a,a_3 (measured by fluorometry and reflection spectrophotometry) became fully reduced during complete ischemia and ECoG was suppressed. Brains were frozen in situ for metabolite analysis at times signalled by the peak hyperoxidation and re-reduction to baseline of cytochrome a,a_3. At hyperoxidation, glucose, glycogen, ATP and CrP were significantly decreased, while lactate was markedly increased. Oxygen consumption was also decreased at this time. At recovery of cytochrome a,a_3 and ECoG, CrP and glucose values were normal, but glycogen and ATP had not changed significantly from the values at peak hyperoxidation. Lactate had fallen but remained significantly greater than control. Neither ADP nor AMP showed significant change at either point.

These data demonstrate that there is residual metabolic dysfunction following cerebral ischemia that may account for continued ECoG suppression despite the presence of oxygen and reoxidation of the mitochondrial respiratory chain. We suggest that hyperoxidation indicates an excess of oxygen relative to reducing equivalent availability. Since NAD and cytochrome a,a_3 both demonstrate hyperoxidation, a block within the respiratory chain itself is unlikely. Since ADP is available to stimulate O_2 consumption, hyperoxidation must be due either to insufficient reducing equivalent supply via glycolysis and the TCA cycle, or possibly to effects on the function of the entire chain from pH shifts. The finding that ECoG recovery occurs with CrP rather than ATP recovery indicates that either trans-synaptic electrical activity is more dependent upon CrP or there is recovery in a specific ATP pool not measureable by present techniques.

This study also demonstrates that sampling of metabolites based upon physiological events, rather than chronological time, provides increased capability for defining effects of ischemia and other perturbations in terms of both electrical and metabolic changes. (Supported by PHS grants NS 05820, NS 14325 and NS 07238).

293.17 CALCIUM- AND CALMODULIN-DEPENDENT PHOSPHORYLATION IN ISCHEMIC BRAIN. C.G. Wasterlain* (SPON: I. Gershon). Dept. of Neurology, VA Medical Center, Sepulveda, CA. 91343, and UCLA School of Medicine.

Calcium-dependent cell death is a common mechanism of ischemic myocardial damage. In the central nervous system, its role in ischemia remains uncertain. In the process of isolating a calcium- and calmodulin protein kinase from brain regions, we were impressed by its lability with increasing dissection time, previously noted by others (Goldenring et al.,J.Biol.Chem.,258:12632,1983). A study of calcium- and calmodulin-dependent phosphorylation indeed revealed major changes in the ischemic brain. Protein phosphorylation was assayed in vitro in synaptic plasma membrane (SPM) using γ-[^{32}P]-ATP as phosphate donor, followed by SDS-PAGE and autoradiography. ^{32}P was incorporated into many proteins, and in the presence of 100 μM calcium and excess calmodulin this incorporation was enhanced, by 10-20 fold into the subunits of calmodulin kinase (M_R 50,000, 58,000, and 60,000) and to a variable extent into many other proteins. In decapitation ischemia the stimulation by calcium and calmodulin of ^{32}P incorporation into SPM proteins from hippocampus or cerebral cortex was reduced within a few minutes, severely curtailed within 15 minutes and vestigial after 30 minutes. Baseline phosphorylation was reduced less severely. After 30 minutes of ischemia, 100 μM calcium + calmodulin inhibited ^{32}P incorporation into proteins of M_R 97,000, 58,000, 50,000 and 43,000. Assays of calmodulin kinase in cortical SPM at 0°C under conditions that minimize protein phosphatase activity, showed a marked reduction of autophosphorylation after 30 minutes of ischemia, while protein phosphatase activity assayed in the presence of kinase inhibitors was indistinguishable from controls. These data suggest that two important changes take place in ischemic tissue: calmodulin kinase is rapidly inactivated, and calcium in concentrations which are normally stimulatory can inhibit ^{32}P incorporation. These changes may contribute to the symptomatology of cerebral ischemia and to the generation of post-ischemic changes. Supp. by V.A. Res. Service.

293.18 NEUROCHEMICAL ABNORMALITIES IN BRAIN HYPOXIA: REVERSAL BY NOOTROPICS AND OTHER DRUGS. J.D.Hirsch and I.-S Shieh*# Lederle Labs, Pearl River, NY 10965 and #G.D.Searle & Co., Skokie, IL 60077.

The brain is very sensitive to hypoxia which provokes many neurochemical abnormalities.We induced hypoxia in mice by injection of $NaNO_2$ and studied whether any neurochemical effects were modified by nootropics and other drugs. Twenty min.after 125 mpk ip of $NaNO_2$, brain lactate went up by 74% while brain glucose levels were unchanged.Lactate dehydrogenase activity declined by 19% while pyruvate kinase activity increased by 11%.Hexokinase activity was unchanged. Receptor binding of ^{3}H-Ro5-4864 and ^{3}H-dihydromorphine in frontal cortex membranes declined by 22 and 36% respectively while striatal binding of ^{3}H-spiperone went up by 27%.The latter effect was due to an increase in receptor number. Binding of ^{3}H-diazepam, muscimol, QNB, p-aminoclonidine, dihydroalprenolol, and DADLE in frontal cortex membranes was unchanged 20 min after $NaNO_2$.Two hr after 125 mpk ip of $NaNO_2$, brain lactate remained elevated by 36% while glucose levels were up by 84%.Pyruvate kinase activity was still increased by 8% while hexokinase activity was up by 15%. Lactate dehydrogenase activity was identical to controls at 2 hr.Although unchanged at 20 min,^{3}H-nitrendipine binding in frontal cortex declined by 19% 2 hr after $NaNO_2$.To evaluate the effects of drugs in hypoxia, mice were given 125mpk ip $NaNO_2$ followed 60 min later by ip doses of the test drug. Thirty min later mice were killed.Drugs with ED_{50}s(mpk ip) for completely reversing lactate increases were:piracetam (20), aniracetam(75), pyritinol(100), nifedipine(1), and physostigmine(0.5).Pentobarbitol(25 mpk) and diazepam(5 mpk) also completely reversed the lactate increase. 4-Aminopyridine(0.1 mpk) and morphine (5 mpk) reversed the lactate increase by 65 and 52% respectively.Glucose (3600mpk), amphetamine(2 mpk) and CI911(3.2-100 mpk) had no effect on lactate in hypoxia.In controls, CI911 increased lactate (ED_{50} 15 mpk) while the other drugs were inactive.Piracetam and aniracetam could not prevent decapitation-provoked increases in brain lactate, but piracetam at 560 and 1000 mpk ip given before hypoxia induction with $NaNO_2$ completely reversed the increase in striatal ^{3}H-spiperone binding.

These results suggest that reversal of hypoxia-induced neurochemical abnormalities may be useful for evaluating drugs with brain-protective properties.

293.19 REVERSAL OF INDUCED ISCHEMIC NEUROLOGIC DEFICIT IN GERBILS BY L-CYCLOSERIN, AGONIST AND ANTAGONIST OF BENZODIAZEPINES (BZD) RECEPTORS. G. Delbarre, B. Delbarre and A. Ferger*. Lab. Chir. Exp., Faculté de Médecine, 37032 TOURS, FRANCE

The levels of GABA in the ischemia cerebral cortex of gerbils increase after ligation of one common carotid artery (W.D. Lust et al., Brain Res., 98 : 394, 1975). But, no pharmacological data are available on role of GABA in cerebral ischemia. To investigate this action, we have used drugs known to interfere with levels of GABA (L-Cycloserin) and facilitation of GABAergic transmission (BZD agonist : chlordesmethyldiazepam -C.D.D.- and antagonists : RO 151788, Flumazepil and RO 153505).

The experiments were carried out in adult Mongolian gerbils (60-80 g) of either sex. Mongolian gerbils were selected and only positive animals (which respond immediately by ptosis resulting from transitory interruption of carotidian supply) were used in the experiment. They were anaesthetized with ketamine (50 mg/kg - IP) and groups of 8 animals were constituted :

- control group : saline was administered
- treated group : L-Cycloserin, in contrast to D-Cycloserin, was found to inhibit pyridoxal phosphate dependent enzymes, but to have a particular high affinity to the GABA degrading enzyme GABA-transaminase (GABA-T). Consequently, L-Cycloserin raised the GABA concentration. Antagonists of BZD, RO 151788,(Flumazepil) and RO 153505, agonist of BZD, chlordesmethyldiazepam (C.D.D.).

Neurological status was evaluated with the stroke index reported by McGraw et al. (Stroke, 7 : 485, 1976) starting one hour after ligation and again at 4, 24, 48 and 72 hours.

Saline	RO 151788 1 mg/kg per os (T - 30 min)
21,75 ± 5,98	0,75 ± 0,36 **
Saline	RO 153505 3 mg/kg per os (T - 30 min)
26 ± 5,23	6,42 ± 4,53 *
Saline	L-Cycloserin 30 mg/kg per os
22 ± 5,85	9,25 ± 5,41 * (T - 180 min)
Saline	C.D.D. 1 mg/kg per os (T - 90 min)
20,28 ± 4,39	9,25 ± 3,69 *

Mean Stroke index at 72 hours.
Significantly different from saline control $P < 0,05$*
n = 8 $P < 0,005$**
MANN and WHITNEY U. TEST

These results suggest that benzodiazepine GABA chloride ionophore receptor complex may be also involved in mechanism of cerebral ischemia.

293.20 TOWARD THE MECHANISM OF ELECTRICAL FAILURE DURING ANOXIA, IN HIPPOCAMPAL SLICES. J.G. Sick, E.L. Solow, S.M. Pikarsky and M. Rosenthal. Dept. of Neurology, Univ. of Miami, Miami, FL 33101.

To define the mechanism(s) underlying loss of electrical excitability in hippocampal slices of rat brain during anoxia, simultaneous measurements were made of: a) extracellular field potentials and extracellular potassium ion activity (K^+_o) with double-barreled ion sensitive microelectrodes; and b) reduction/oxidation (redox) status of mitochondrial electron transport carriers by transmission rapid-scanning spectrophotometry. Prior to decapitation and slice preparation, the brain was perfused with oxygenated artificial cerebrospinal fluid (ACSF) to removed all blood. The K^+ sensitive microelectrode was positioned in the stratum pyramidale of hippocampal field CA1 to record both the population action potential elicited by electrical stimulation of the Schaffer collaterals and changes in K^+_o.

As expected during normoxia, K^+_o was 3 mM, the population spike was 5-10 mV in amplitude, and there was a redox gradient measured from cytochrome b to c to a,a_3. When anoxia was induced by switching the humidified gas mixture from 95% O_2 - 5% CO_2 to 95% N_2 - 5% CO_2, the redox gradient was abolished within 10 sec as all cytochromes became reduced. The population spike amplitude was depressed within 30 sec, and abolished within 60 sec. At this time, K^+_o was elevated only to less than 2 mM above baseline, indicating that cells in the vicinity of the potassium sensitive electrode had not completely depolarized despite loss of the population spike. This interpretation was supported by continued recordings of the presynaptic Schaffer collateral pre-volley after the post-synaptic population spike had disappeared. Within 3-5 min following anoxia, K^+_o was elevated to greater than 30 mM, indicating cell depolarization. At this time, the Schaffer collateral pre-volley also disappeared. These data indicate that synaptic transmission failed previous to cell depolarization during anoxia, possibly through oxygen sensitive processes in the synapse. This failure of synaptic transmission may serve as a protective mechanism under conditions when energy production is limited. (Supported in part by PHS grants NS17945, NS05820 and NS14525).

293.21 PHARMACOLOGICAL PRETREATMENT OF SECONDARY ISCHEMIA IN HIPPOCAMPAL BRAIN SLICES J.L. Parmentier and M.D. Taylor*, Anes. Res. Lab., Univ. South Alabama, Mobile, AL 36688.

The multiple etiology and unpredictability of cerebral ischemia effectively precludes its pharmacological pretreatment. Primary stroke management thus involves the restoration of blood flow to viable neurons in the penumbra of infarcted areas. However, sudden reoxygenation can initiate a secondary ischemic condition which involves, in part, a complex series of chemical reaction cascades which are themselves deleterious to cerebral tissue. We have studied functional correlates of secondary reflow damage in rat hippocampal brain slices made anoxic by the replacment of oxygen with nitrogen in an "Oslo" chamber. Alternate orthodromic and antidromic stimulations were delivered to CA1 pyramidal cells while recording extracellularly from stratum radiatum and stratum pyramidale. EPSP and population spike amplitudes recover from 6-8 minutes of anoxia, but not from a 10 minute insult. EPSPs following orthodromic stimulation are lost under hypoxia before prevolleys and the antidromically stimulated population spike are eliminated. Methylprednisilone (MP) (10^{5} M), a steroidal antiinflammatory agent known to block phospholipase A2 activity, protects synaptic transmission from 10 minutes of anoxia provided the drug is in the tissue when anoxia begins. Protection is not conferred if MP is delivered during reoxygenation. Indomethecin, a blocker of cyclooxygenase, and allopurinol, a blocker of xanthine oxidase, also protect transmission, though to a lesser degree. These observations are consistent with the oxygen-induced free radical hypothesis of secondary ischemic damage. Methylprednisilone, and other steroids, prevent the release of free fatty acids from membrane lipids. This effect works to limit the availability of substrates for the burst of metabolism which accompanies reintroduction of oxygen to ischemic tissue. Thus less structural damage occurs to membrane lipids and fewer fatty acids, particularly arachidonate, are available to follow synthesis pathways which generate free radicals. This stabilization effect by the steroids also preserves the function of proteins which are embedded in the lipid membrane. Other drugs which will inhibit synthesis pathways that generate free radicals, or which act as free radical scavengers should also provide protection from the hypoxic-ischemic conditions of secondary reflow.

293.22 EFFECTS OF TEMPERATURE ON SYNAPTIC TRANSMISSION IN HIPPOCAMPAL TISSUE SLICES. S.J. Schiff. Division of Neurosurgery and Department of Physiology, Duke University Medical Center, Durham, NC 27710.

350 μm rat hippocampal slices were fully submerged in 29°C artificial cerebrospinal fluid (ACSF) flowing at 2 ml/min. Responses to Schaffer collateral stimulation were recorded in the stratum radiatum and stratum pyramidale of the CA1 region. At 90, 120, 150, and 180 min following slice preparation, input/output (I/O) curves were constructed by averaging 4 responses to each of a range of stimulus intensities. Temperature was held within 1°C during controls. During experiments, the chamber was warmed and then cooled between the 90 and 180 min recordings. Warming the chamber to 33°C for 15 min produced an increase in the afferent fiber volley (prevolley) and field-excitatory postsynaptic potential (fEPSP), and a marked decrease in the amplitude of the population spike. Cooling the chamber following 45 min at 33°C produced complete reversal of these effects. Heating the chamber to 37°C resulted in an enhancement of the effects seen at 33°C; however, complete reversibility was not seen, despite maintaining this higher temperature for only 15 min. Changes after cooling consisted of a depression in prevolley amplitude and fEPSP initial slope.

Between I/O collections, the slice was continuously stimulated at 0.1 Hz at a submaximal intensity. This permitted observation of the dynamic response of the reflex output as the temperature was changed. Warming the slice produced a subtle depression in the average population spike amplitude for 2-3 min, followed by a 2-4 min increase in amplitude that may be dramatic when heating the chamber to 37°C. This increase in amplitude may be accompanied by double population spikes, and is followed by rapid decay of spike amplitude over 10 min to produce the steady state effects noted above.

The combination of larger prevolley and fEPSP with smaller population spike seen in warm compared to cool slices in the steady state may reflect a hyperpolarization of both presynaptic terminals and postsynaptic neurons prevailing at higher temperatures. The magnitude of the transient increase in excitability seen during a temperature increase is related to the rate of temperature rise; although not readily explainable, perhaps a momentary increase in transmitter release or effect is operative here.

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293.23 CEREBRAL METABOLIC EFFECTS OF EXPERIMENTAL HEAD TRAUMA. P.A. Tornheim, M.K. Eichold* and K.R. Wagner. Depts of Anat. & Cell Biol. and Neurol., Univ. of Cincinnati Coll. of Med., V.A. Med. Center, Cincinnati, OH 45267

We recently observed very early (15 minute) changes in cerebral metabolite concentrations following blunt head injury in the cat. In the present study, regional cerebral metabolites and brain edema were measured at 2 and 6 hours after injury to determine the time course for traumatically-induced metabolic changes and their relationship to cerebral edema.

Physiologically-monitored anesthetized cats were impacted on the left side of the exposed skull with a Remington Humane Stunner. Control cats were sham injured. Brain metabolite concentrations were fixed by in situ freezing. Head-injured cats without brain tissue hemorrhage and those with unilateral cerebral contusions were selected for study. Regional white matter and cortical samples were measured for metabolites by enzymatic-fluorometric methods. Brain edema was quantitated with an organic gradient; vasogenic edema was evaluated with vascular Evans Blue dye. Metabolite concentrations were corrected for dilution with edema fluid.

Head-injured cats without contusion had small metabolic perturbations in the cerebral cortex on the side of impact at 2 hours after trauma, with return of metabolites to control levels by 6 hours after the injury.

In cats with unilateral contusion, the cerebral cortex neighboring and contralateral to contusion had a substantial increase in lactate and decreases in high energy phosphates at 2 hours after injury, with metabolic improvement to values near control levels by 6 hours. At both 2 and 6 hours after injury, the white matter within the territory of vasogenic edema of contusion had a very large increase in lactate and a decrease in ATP; white matter peripheral to the edema front had a modest increase in lactate without alterations in ATP or phosphocreatine.

Cortical edema observed with this model correlated well with changes in cerebral metabolites, suggesting a metabolic component to the cortical edema of head injury. We speculate that the data from white matter suggest acute white matter metabolic perturbations that are exaggerated by the spread of vasogenic edema from areas of contusion. Our findings contrast with those of the cold injury and lead to the conclusion that prolonged metabolic dysfunction occurs in the white matter surrounding areas of traumatically-induced tissue hemorrhage.

This study was supported by NIH Grant #R01 NS17975.

293.24 DISTRIBUTION OF PYRUVATE DEHYDROGENASE IN RAT BRAIN: SOME REGION-SELECTIVE CHANGES IN TWO EXPERIMENTAL MODELS OF THIAMINE-DEFICIENCY ENCEPHALOPATHY. Roger F. Butterworth, Jean-François Giguère* and Anne-Marie Besnard*. Lab. of Neurochemistry, Clinical Research Centre, Hôpital Saint-Luc, Montreal H2X 3J4.

It has been suggested that decreased activity of the thiamine-dependent pyruvate dehydrogenase complex (PDHC) may play a major role in the pathophysiology of thiamine-deficiency encephalopathy. PDHC was measured in homogenates of 14 regions of the rat brain using an arylamine acetyltransferase coupled assay in which the enzyme complex is completely activated [Ksiezak-Reding et al., J. Neurochem., 38, 1627 (1982)]. PDHC activities in the rostrally-situated brain regions (hippocampus, cerebral cortex, striatum) were found to be 1.5 to 2.5 times higher than those of the caudally-situated regions (cerebellum, pons, medulla).

Daily administration of pyrithiamine (0.5 mg/Kg, i.p.) for 15 days leads to neurological signs of thiamine deficiency including catalepsy, loss of righting reflex and convulsions. Measurement of PDHC in 11 regions of the CNS of symptomatic pyrithiamine treated (PT) rats revealed no significant abnormalities when the activity of the enzyme complex was measured in the absence or presence of added thiamine pyrophosphate (TPP). On the other hand, rats made symptomatic by chronic thiamine deprivation showed moderate region-selective decreases of PDHC activity. Decreases (of the order of 20-30%) were confined to midbrain, pons and lateral vestibular nucleus, three regions of brain generally associated with pathological damage in thiamine-deficiency encephalopathy. The observed region-selective decreases in PDHC were partially restored to normal by the addition of TPP in vitro. Such changes may be responsible for the neurological impairment observed following chronic thiamine deprivation and these findings again underline the differences between these two experimental procedures used in the study of thiamine-deficiency encephalopathy. [This study was funded by The Medical Research Council of Canada. We thank La Fondation Georges Phenix (University of Montreal) for a studentship (to J.F.G.)]

294.1 CARBACHOL INFUSIONS IN RATS: "BRAKING" OF NATRIURESIS AND ABSENCE OF SALT APPETITE. D.A. Fitts*, R.L. Thunhorst*, and J.B. Simpson. (SPON: D.M. Bowden). Dept. of Psychol., Univ. of Washington, Seattle, WA 98195.

Intracerebroventricular (IVT) infusions of carbachol (CBC) or of angiotensin II (AII) both produce natriuresis but apparently only AII stimulates salt appetite. The AII-induced appetite includes two components: an early phase (3-10 hr), occurring prior to any natriuresis, and a later phase which may be secondary to natriuresis. CBC does not provoke an appetite comparable to the early phase of the AII-induced effect. However, it is unclear if injections or infusions of CBC caused sufficient Na loss to produce a salt appetite similar to the later phase of the AII-induced appetite. The present studies measured both Na excretion and Na intake during 6-hr acute and 6-day chronic CBC infusions into the lateral ventricles of rats (n=28 in each study). Acute infusions of vehicle, 400, or 2000 ng/hr CBC produced dose-dependent increases of water intake and Na excretion, which were maximal during the first 2 hr and declined to control levels by 4-6 hr. Cumulative Na excretions during the 6 hr for the vehicle, 400, and 2000 ng doses were: 387±308; 1583±593; and 2795±821 mmol (mean±S.D.). The drug-induced losses represent an estimated 15-30% of extracellular Na. Salt appetite did not appear during the 6 hr. Chronic, 6-day lateral ventricular infusions of vehicle or 2000 ng/hr CBC via minipumps produced a chronic elevation of water intake, but no increase of 0.45 M NaCl solution consumption. The Na balances of CBC-infused rats maintained on low Na diet with 0.45 M NaCl and water to drink were negative during the first day, but gradually returned to normal over 6 days, despite the absence of any increase in Na solution consumption. Plasma volume did not differ between CBC- and vehicle-infused rats on day 6 either in groups maintained on low Na or normal diets with saline available for drinking. A 10% decline in plasma volume occurred in the low Na diet groups, regardless of drug treatment, suggesting that Na excretion during extended CBC infusion achieved a level appropriate to the dietary Na. Plasma Na and K concentrations were normal in CBC-infused rats by day 6. Cholinergic stimulation of the brain thus appears to interfere with the usual salt appetite following Na loss, while the Na deficit induced by the initial natriuresis was corrected via means other than ingestion.
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294.2 SALT APPETITE IS SUPPRESSED BY CARBACHOL AND FACILITATED BY ANGIOTENSIN II FOLLOWING FUROSEMIDE DIURESIS. R.L. Thunhorst*, D.A. Fitts*, and J.B. Simpson. (SPON: T.T. Kennedy). Dept. of Psychol., Univ. of Washington, Seattle WA 98195.

Prolonged intracerebroventricular (IVT) infusions of angiotensin II (AII) provoke salt appetite, but infusions of carbachol (CBC) do not, even following an estimated 30% of extracellular volume loss owing to CBC-induced natriuresis. The present study examined the influences of IVT infusions of 80 and 400 ng/hr of CBC, of 88 and 438 ng/hr of AII (5:1 molal ratio of CBC:AII), or saline vehicle on water and saline consumption following 25 mg/kg furosemide, ip. Thirty-three rats were administered the furosemide and placed into urine collection cages for 4 hr without food or fluids for ingestion. Neither the mean urine volumes of 16-19 ml/4 hr nor the Na excretions of 2.0-2.3 mmol/4 hr differed among the 5 groups during the diuresis. Infusions of 1 µl/hr were begun after the urine collection, and both water and 0.3 M NaCl solution were given 15 min later. Water drinking commenced 2-9 min later, while saline drinking occurred within 10-50 min. Vehicle-infused rats consumed 6.6±2.0 ml of water and 2.5±2.0 ml saline in 4 hr (mean±S.D.). Water consumption was elevated in all drug-infused groups, accumulating an average 9.7 and 11.8 ml at the lower doses and 31.2 and 16.7 ml at the higher doses of AII and CBC, respectively. Saline intake, however, showed a dose-dependent increase with AII, to 4.4±3.9 and 9.0±5.1 ml/4 hr, but a decrease with CBC, to 2.0±1.5 and 0.7±1.1 ml/4 hr. Sodium balance during the 4 hr of infusion was largely dependent on sodium intake: the vehicle-infused and 80 ng/hr CBC-infused groups both replaced about 25%, while the AII-infused groups replaced 50-100% of the furosemide-induced Na loss. The 400 ng/hr CBC group failed to replace Na, and further reduced Na balance by 0.25 mmol during the infusion. Nevertheless, all drug-infused groups increased water balance, from 7.0±2.0 ml in the vehicle-infused, to 12-30 ml in the AII and CBC groups. The high water balance (15 ml) and negative Na balance (-0.25 mmol) during the infusion at the higher dose of CBC thus indicates considerable dilution of body fluids without replacement of extracellular volume. The experiment demonstrates that cholinergic stimulation of the brain effectively disrupts normal extracellular volume regulation, while AII facilitates extracellular volume replacement.
Supported by HL 21800.

294.3 FLAVOR, FORCED CHOICE AND DEPRIVATION AFFECTS CORTICOSTERONE SELECTION BY ADRENALECTOMIZED RATS. V. Willer*, T.W. Castonguay*, M.F. Dallman* and J.S.Stern* (Spon: B. Wong). Food Intake Laboratory and Nutrition Dept. Univ. Ca. Davis 95616 and Physiology Dept., UCSF, San Francisco 94143.

Adrenalectomized (adx) rats given access to saline solutions containing corticosterone (B) maintain normal body weights (Wilkinson et al., Am. J. Physiol., 1981). The purpose of this experiment was to determine if adx rats can learn to prefer a flavored saline with B over a different flavor without B.

The experiment was conducted over 22 days. The experimental design consisted of two bottle preference tests (flavored saline with B vs. flavored saline without B) alternating with single bottle tests (forced choice either with or without B). Male, adx Sprague Dawley rats (12 wks old) were given either B in anise flavored (n =6) or in maple flavored (n =5) saline for 6 days. During the two-bottle preference tests, each rat also received unsupplemented saline in the alternate flavor as the second option. This first two bottle preference test was followed by: a 4-day forced choice test, during which the rats only drank the flavored saline with B; a 4-day two bottle preference test; a 4-day test of flavored saline without B (i.e. deprivation); and finally a 4-day two bottle test.

During the first choice period, the MAPLE group consumed a daily average of 36.2 ± 5.8 ml of saline with B. The ANISE group consumed only a daily average of 7.0 ± 4.2 ml. of saline with B. Despite access to B, rats in the ANISE group were essentially B deprived because they were consuming the preferred, but unsupplemented maple flavored saline. During the forced choice period there was an increase in B intake only in the self-deprived group (the ANISE group 22.6 ± 3.8 ml but not the MAPLE group 26.5 ± 7.0 ml). The rats in the MAPLE group did not experience deprivation until the deprivation period was imposed. After deprivation, B intake increased in both groups.

Results from the first choice period show that flavor preferences can override any tendency to consume B. However, both forced choice and deprivation can promote an increased preference for a B containing solution. The fact that there was a selective increase in B consumption with both forced choice (in the ANISE group) and deprivation (in both groups) demonstrates that adx rats can acquire a preference for B.

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294.4 THE ROLE OF OROPHARYNGEAL STIMULATION IN CCK-INDUCED SATIETY IN THE SHAM FEEDING RAT. P.A. Forsyth*, S.M. Collins*, K.L. Conover* and H.P. Weingarten. Departments of Psychology & Medicine, McMaster University, Hamilton, Ontario, Canada, L8S 4K1.

It is generally believed that oropharyngeal stimulation potentiates the satiety produced by exogenous cholecystokinin (CCK). This belief is based on experiments using 20% pure CCK which demonstrate that CCK's ability to suppress feeding is enhanced the closer it is injected to a meal. However, the increased efficacy of CCK with closer temporal proximity to a meal might simply reflect increased peptide levels at the time of feeding. Further, since oropharyngeal synergy has never been demonstrated with a pure CCK preparation, the following studies were performed to evaluate the role of oropharyngeal stimulation in CCK-induced satiety.

Rats equipped with gastric fistulae were injected ip with 5.6 ug/kg CCK-OP 15 min before a test sham feed. In one condition, rats sham fed for 15 min prior to CCK injection; in the other, they did not. CCK-OP given 15 min before sham feeding suppressed eating in only those cases when its administration was accompanied by oropharyngeal stimulation. These results indicate that oropharyngeal cues enhance the satiety action of exogenous CCK.

A second experiment examined whether oropharyngeal synergy with CCK requires oropharyngeal stimulation prior to peptide delivery. CCK-OP, 5.6 ug/kg. was injected into rats coincident with the initiation of a test sham feed. Rats had either sham fed, or not sham fed, for 15 min prior to CCK administration. Both conditions produced similar and significant suppressions of eating during the test sham feed: the maximum suppression, time of peak suppression, and duration of suppression were similar in the two CCK conditions. Thus, although oropharyngeal cues do enhance the action of exogenous CCK, this oropharyngeal synergy needs only contiguous pairings of oropharyngeal stimulation and feeding, and does not require oropharyngeal stimulation prior to CCK injection.

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294.5 EFFECT OF GASTRIC DISTENSION ON SINGLE-NEURON ACTIVITY IN THE BRAINSTEM OF THE RAT. L.A. Evey, and J.C. Mitchell. Dept. of Psychology, Kansas State Univ., Manhattan, KS 66506.

Neuroanatomical evidence indicates that gastric vagal afferents terminate in the caudal medial portion of the solitary nucleus whereas gastric vagal efferents arise from somata in the underlying portion of the dorsal motor nucleus of the vagus nerve (Norgren and Smith, Neurosci. Abst., 1983, 9, 611). We used neurophysiological techniques to further investigate the central distribution of gastric afferents and the response characteristics of single neurons sensitive to gastric distension. Sprague-Dawley rats (n=28) were anesthetized with Chloropent (3.0ml/kg) and tracheotomized. Rats were mounted in a stereotaxic apparatus and the dorsal brainstem exposed. Supplemental doses of chloralose (55mg/kg) were given as required. Recordings were from 26 neurons sensitive to inflation (10ml air) of a gastric balloon. Responsive cells were within 0.6mm of the midline and were at depths from 0.6mm to 1.0mm from the surface of the brain. They extended from 1.0mm posterior to the obex to the edge of the cerebellum. Whether they extended further rostrally was not investigated. Histological verification of microlesions located these cells in an area immediately subjacent to the area postrema and the caudal medial solitary nucleus near the border of the dorsal motor nucleus of the vagus nerve. It is inconclusive whether recordings were from second-order cells within the solitary nucleus or higher-order cells within the motor nucleus of the vagus. Extracellular spike amplitudes were as high as 4500uv and always showed an inflection on the rising edge suggesting that recording sites were in the immediate vicinity of cell bodies. Spontaneous firing rates ranged from less than one spike per minute to approximately one spike per second. Nine cells increased firing rates to distension and decreased firing rates to deflation. Seventeen cells reduced firing rates to distension and increased firing rates to deflation. These responses are similar to the Type I and Type II units from vagal efferents previously described by Davison and Grundy (J. Physio., 1976, 263, 219P-220P). Neurons showing Type I responses were located immediately dorsal to neurons showing Type II responses. We agree with previous researchers that these neurons mediate gastric reflexes and may be involved in satiety.

294.6 IS GASTRIC SHAM-FEEDING REALLY SHAM FEEDING? A. Sclafani and J. W. Nissenbaum*. Dept. of Psychology, Brooklyn College of CUNY, Brooklyn, NY 11210.

Rats fitted with a gastric fistula drastically increase their food intake when tested with their gastric fistula opened. This sham-overfeeding response has been attributed to the action of positive feedback from the mouth (palatable taste) in the absence of negative feedback from the gut (satiety cues). The present study confirmed that hungry rats increased their intake of a 32% glucose solution when tested with an opened gastric fistula (closed fistula: 7.8 ml/30 min, opened fistula: 35.1 ml/30 min). The same rats, however, failed to reliably increase their intake of a 0.2% saccharin solution when tested with an opened fistula (closed fistula: 11.5 ml/30, opened fistula: 16.2 ml/30 min). The rats also did not sham-overfeed saccharin solutions of higher or lower concentrations (.05 to .8%). The bitter aftertaste of saccharin cannot explain these results because other rats were observed to sham-feed considerably less of the saccharin solution than of a polysaccharide solution made bitter with sucrose octaacetate.

The above findings suggest that postingestive factors rather than oral factors alone may be responsible for the sham-overfeeding of carbohydrate solutions. Further tests revealed that the sham-feeding of concentrated glucose or sucrose solutions significantly increased blood glucose level. The addition of acarbose, a drug that blocks sucrose digestion, to the sucrose solution prevented the increase in blood glucose produced by sham-feeding. This indicated that absorption rather than endogenous release was responsible for blood glucose response to sham-feeding. Since acarbose did not reduce the sham-overfeeding of the sucrose solution it appears that postabsorptive factors are not responsible for this behavior. Rather the results suggest that preabsorptive afferent feedback from the gut, presumably from the gastric mucosa, is responsible for the sham-overfeeding of carbohydrate solutions. This possibility is currently under investigation.

294.7 ATROPINE INHIBITION OF SHAM FEEDING: TEMPORAL FACTORS. J. W. Nissenbaum* and A. Sclafani (SPON: I. Abramov). Dept. of Psychology, Brooklyn College of CUNY, Brooklyn, NY 11210.

Treatment with atropine methyl nitrate has been reported to substantially suppress the sham-feeding of a liquid diet in rats (Lorenz et al., 1978). On the other hand, much higher doses of atropine were observed to produce relatively small reductions in the real-feeding of sugar solutions (Sclafani & Xenakis, 1982). The present study investigated the reason for this discrepancy using rats fitted with a gastric fistula. In Exp. 1, rats trained to sham-feed a 32% sucrose solution were injected with atropine (1 or 5 mg/kg) or saline 30 min prior to the sham-feeding test following the procedure of Sclafani & Xenakis. Atropine suppressed the sham-feeding of sucrose by up to 16% which is comparable to the results obtained with rats "real-feeding" a sucrose solution (Sclafani & Xenakis). In Exp. 2, the rats were tested according to the procedure of Lorenz et al. That is, atropine (1 or 5 mg/kg) was injected 17 min after the rats had started sham-feeding. In this case atropine suppressed the subsequent sham-feeding of the sucrose solution by up to 65%. In Exp. 3, the atropine (1 mg/kg) was injected at the start of the sham-feeding test and produced a feeding suppression similar to that observed in Exp. 2.

These results demonstrate that atropine does not differentially affect the real-feeding and sham-feeding of a sucrose solution. They reveal instead that the temporal relationship between drug administration and meal onset is a critical determinate of the drug response. When the drug is given 30 min prior to meal onset atropine produces only a small suppression of feeding, but when the drug is administered at or after meal onset it produces a large depression in food intake. This temporal effect cannot be attributed to a short duration of action for atropine, but rather appears to result from some interaction between the initial effect of the atropine and feeding behavior.

294.8 INSULIN'S INFLUENCE ON SHAM VS. NORMAL FEEDING. Rhonda L. Oetting* and Dennis A. VanderWeele. Dept. Psych., Occidental College, CA 90041.

Insulin administered in doses above physiological levels produces hyperphagia and, with continued use, obesity. Alternatively, insulin infused chronically in more modest amounts decreases food intake and body weight gain. The present study continued to examine insulin's function in the control of food intake by observing the effects of insulin in animals 17½ hrs food deprived either feeding with open gastric fistulas (sham) or feeding normally.

Seven adult male Sprague-Dawley rats were surgically implanted with stainless steel gastric fistulas sutured into the wall of the stomach along the greater curvature. Opening the fistula allowed immediate drainage of stomach contents. 17½ hr food deprived rats received injections of insulin (.1,.4 or .75 U/rat) or saline on alternate days, prior to a 1 hr presentation of a liquid sweetened condensed milk solution. In sham feeding animals, insulin significantly reduced intake during the testing period at the .4 U dose (18%, $p<.02$) and .75 U dose (11%, $p<.05$). A subsequent study which paired flavored milk with insulin and alternatively flavored milk with saline, indicated that the suppression was not due to illness as the insulin-paired flavor was preferred in two-bottle taste tests ($p<.02$).

Eight additional rats were used to test insulin's effects on normal feeding. Subjects (17½ hr deprived) received counterbalanced injections of insulin (.05,.1,.4 or .75U/rat) or saline over five consecutive days. Slight suppressions of intake that were not statistically significant (approximately 6% suppression) were observed in normally feeding rats at the doses given.

The results indicate that insulin reduces intake in sham feeding rats with doses that produce nonsignificant trends to decrease real intake. Gastrointestinal hormones such as CCK and bombesin require higher doses to suppress sham intake than real intake. Perhaps insulin reacts in a similar manner, with lower doses required to reduce normal intake than those doses necessary to decrease sham intake. This possibility is currently being investigated.

294.9 EFFECTS OF PARTIAL LIVER DENERVATIONS ON FEEDING AND DRINKING RESPONSES IN RATS. L. MacIsaac*, M. Esguerra*, and N. Geary. Department of Psychology, Columbia University, New York, NY 10027.

Although effects on food and water intake of total liver denervation[1] and of selective vagotomy of only the hepatic branch of the vagus[2,3,4] have been tested, effects of partial liver denervations that spare the hepatic branch have not. We therefore investigated ingestive responses that may depend on hepatic function in rats with selective hepatic vagotomies (HV), selective liver denervations that spare the hepatic branch of the vagus (PLD), and sham operations (SH). Operative procedures were based on published methods[1,2].

Pancreatic glucagon (100-400 mcg/kg ip) inhibited feeding in both SH and PLD rats (mean inhibitions of meal size compared to control injection:SH, 18-24%; PLD, 31-35%), but failed to inhibit feeding in HV rats (inhibition:-10 - -8%). In contrast, neither neural disconnection reduced epinephrine's (12.5-50 mcg/kg ip) inhibitory effect on feeding (inhibitions: SH, 28-41%; HV, 10-55%; PLD, 26-61%). These data suggest different peripheral neural mechanisms mediate the effects of glucagon and epinephrine on feeding. They also fail to support the hypothesis that coeliac ganglionectomy attenuates epinephrine's inhibitory effect on feeding by disconnecting hepatic afferents[3]. Why total liver denervation does not block glucagon's satiety effect[1] remains mysterious.

Three dipsogenic challenges failed to reveal any effects of HV or PLD. Latencies to drink and water intakes after 17 h water deprivation, hypertonic saline injection (1 ml/kg 1 M NACL ip), or angiotensin II injection(0.1 mg/kg sc) were similar in SH, HV, and PLD rats. Because coeliac vagotomy produces deficits in both hypertonic saline and angiotensin induced drinking[4,5], this suggests a further dissociation between the behavioral effects of liver denervations and coeliac lesions.

REFS: [1]L. Bellinger & L. Williams Physiol. Behav. 30: 436, 1983. [2]N. Geary & G.P. Smith Physiol. Behav. 31: 391, 1982. [3]M.G. Tordoff et.al. J. C. C. Psychol. 96: 361, 1982. [4]M.G. Tordoff et.al. Physiol. Behav. 28: 417, 1982. [5]C. Jerome & G.P. Smith J. Autonom. N. S. 9: 259, 1983.

294.10 HEPATIC VAGOTOMY COMBINED WITH SUCROSE SUPPLEMENTATION YIELDS INCREASED BODY WEIGHT IN RATS. L.E. Goehler* and D. Novin. Department of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

The hepatic branch of the vagus nerve has been implicated in body weight control (Friedman and Sawchenko, Am. J. Physiol., in press). That study showed that cutting this branch increased body weight in male but not female rats when fed ad libitum. This finding is not universal, however, as some workers have failed to find either increased body weight gain or altered responses to metabolic challenges (Tordoff, Hopfenbeck and Novin, Physio. Behav., 1982: 28, 417-424). Since control of food intake is unlikely to be the province of one organ we suspected that the information carried by the hepatic vagus may be related to relatively more specific mechanisms of regulation. In particular, the liver is suspected of being involved in carbohydrate regulation, therefore the manipulation used in this study involved the ad libitum dietary supplementation of a 30% sucrose solution. Twenty-four male albino rats (Charles River CD strain) were given either hepatic vagotomies or sham operations. One half of each group received a 30% sucrose supplementation. In addition, all groups had laboratory chow in pellet form continuously available. Only the group receiving both hepatic vagotomy and sucrose supplementation (n=6) demonstrated significant body weight gain relative to sham operated controls not receiving sucrose. Hepatic vagotomy without sucrose supplementation or sucrose supplementation without hepatic vagotomy yielded body weights not different from controls during the 4 week long observation period. These results are not entirely like the results of Friedman and Sawchenko.

These results support the idea that there are receptors for carbohydrate metabolism in the liver. Furthermore, these receptors are likely to be involved in the regulation of food intake and they are, at least in part, innervated by the hepatic branch of the vagus nerve.

294.11 EFFECT OF DIET CHOICE ON FOOD INTAKES OF VAGOTOMIZED RATS. Nancy J. Kenney and Michelle Muggli*. Department of Psychology, University of Washington, Seattle, WA 98195.

Following total subdiaphragmatic vagotomy, rats decrease food intake and body weight. Vagotomized (vgx) rats demonstrate aversions to novel foods presented during the postsurgery period. Under typical conditions, however, vgx rats are not presented with a new food after surgery but are adapted to their postsurgery food for at least 2 wks prior to vagotomy. In this study, we examine the selection of foods by rats which were adapted to their standard postsurgery food prior to vagotomy.

Sixteen male rats were fed sweetened-condensed milk and 16; pelleted laboratory chow. Eighteen days later, half of the animals in each diet group received total subdiaphragmatic vagotomies. All rats were continued on their presurgery diet for 8 days after surgery. On the next 2 days, all rats were fed both pellets and the milk diet. Food intakes and body weights were measured daily.

Both pellet- and milk-fed vgx rats decreased daily intakes compared to controls after surgery. Total caloric intakes of pellet-fed vgx rats and of sham-operated rats did not change on the test days when both foods were available. Total intakes of the milk-fed vgx rats increased ($F(1,7)=13.6$, $p<.01$) but remained lower than those of milk-fed controls ($F(1,17)=7.8$, $p<.05$) when pelleted food was presented in addition to milk.

On the test days when both milk and pellets were provided, milk-fed vgx rats took only 11.8 $\pm$ 4.8% of their total calories from their standard milk diet. Milk-fed controls took 46.0 $\pm$ 8.2% of their calories from the milk ($F(1,13)=13.7$, $p<.01$ compared to milk-fed vgx rats). Pellet-fed vgx rats did not differ from their controls in the proportion of calories taken from each of the foods on the first day of diet choice. On the second day, however, pellet-fed vgx rats, like the milk-fed vgx rats, preferred the newly added food and took only 24.1 $\pm$ 5.3% of their total calories from their standard pelleted food ($F(1,14)=7.9$, $p<.05$ compared to pellet-fed controls).

Thus, vgx rats can develop aversions to a diet presented both before and after surgery. This effect is most apparent for animals fed a milk diet in the peri-surgery period but also can be demonstrated for pellet-fed rats under appropriate conditions. Diet aversion, however, does not account for the entire suppression of food intake which follows vagotomy.

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294.12 Food Intake and Meal-patterning following Gastric and Hepatic-portal Infusion of Hexoses in the Rabbit. Paula J. Geiselman, Department of Psychology, UCLA, Los Angeles, CA 90024.

We have previously shown that, when glucose or fructose was rapidly infused into the duodenum or was ingested normally, rabbits showed an increase in subsequent chow intake. Galactose, however, had little effect. We have extended these results to study other possible loci that may mediate the sugar-induced food-enhancement effect.

Female New Zealand rabbits were implanted with either an hepatic-portal cannula or a gastric cannula and were infused (10 ml/3 kg BW) with 0.3M glucose, 0.3M fructose, 0.3M galactose, and 0.15M NaCl delivered at 3 ml/min. Rabbits were also subjected to a mock control procedure.

During the initial half hour following hepatic-portal infusion of each of the hexoses, the mean meal size, mean meal duration, and total food intake were significantly greater than measures obtained in control conditions. However, after the initial 30 minutes postinfusion of each of the three hexoses, food intake was no longer different from control levels. Thus, the food-enhancement effect of hepatic-portal hexose infusions was not so robust as the longer-lasting effects we had found following duodenal infusion or normal ingestion of glucose or fructose.

During the first half hour following gastric infusion of saline, fructose, or galactose, rabbits ingested significantly more food than observed in the mock condition. However, after the first half hour postinfusion of saline, fructose, or galactose, food intake was not significantly different from that observed in the mock condition. No enhancement effect was observed following intragastric infusion of glucose.

We have found that the oral sugar-induced food-enhancement effect was potentiated by vagotomy and are in the process of studying this further in vagotomized rabbits.

Our results have implications for gastrointestinal and metabolic mechanisms that may control food intake.

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294.13 ADAPTIVE DIETARY SELF-SELECTION IN THE INSULIN-TREATED DIABETIC RAT. E. K. Walls*, M. M. Layman*, J. P. Olson* and T. B. Wishart. Department of Psychology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

In the absence of insulin, dietary fats become an important source of calories for diabetics. The use of high fat diets in diabetic rats has been shown to reduce the severity of diabetes symptoms such as polyuria, polydipsia, hyperphagia and lens cataracts. As these important benefits accrue from ingestion of high fat diets, it would be advantageous for a diabetic rat to select such a diet if given a choice. Previous studies (Armstrong, Walls, Wolfs and Clausen, 1982) failed to show a clear preference for a high fat diet over regular chow.

Three groups of twelve rats were maintained on one of three, isocaloric, semipurified diets. Twenty percent of useable calories in each diet were derived from protein. The remaining energy content of each diet consisted of: 60% carbohydrate and 20% fat (LFD); 40% carbohydrate and 40% fat (MFD); 20% carbohydrate and 60% fat (HFD). All rats were made diabetic by intracardiac injection of streptozotocin (55 mg/kg). Following a 48 hour diabetes verification period, rats were maintained for 7 days with one daily 2.0 U dose of Lente insulin. For the remainder of the experiment, 6 rats in each diet group received either 1.0 or 4.0 U of Lente insulin daily.

When given a choice between all three diets (cafeteria feeding), all rats showed a preference for the HFD. In rats initially maintained with LFD or HFD, the low insulin treatment was associated with significantly greater dietary fat intake. This effect was not obtained in the MFD condition. The selection of HFD resulted in significant improvement in metabolic stability as indicated by the water/food ratio.

These results suggest that the severely diabetic rat has the capacity to improve metabolic status by diet selection.

294.14 **NEURAL CONTROL OF THE PANCREATIC ISLET: CONDITIONED FEEDING AND INSULIN SECRETION.** H.-R. Berthoud, M.Sterner*, and T.L. Powley. Lab. OF Regulatory Psychobiology, Purdue Univ., West Lafayette, IN 47907.

When a neutral stimulus is repeatedly paired with food delivery and feeding within the temporal limits required for classical conditioning, the cue becomes a conditioned stimulus (CS) that will increase motor activity (Zamble, JCPP, 63, 526,1967) and cause feeding (Weingarten, Science, 220, 431, 1983). Since it is also possible to classically condition visceral secretions such as insulin (Woods et al., JCPP, 91, 128, 1977) and saliva and gastric acid as well (see Powley, Psychol. Rev., 84, 89, 1977 for discussion), one might predict that classically conditioned feeding would be associated with neurally-mediated conditioned metabolic responses (e.g., insulin secretion) to the CS.

Male Sprague Dawley rats were fitted with chronic jugular vein catheters and trained for at least 12 days to eat 5 to 6 liquid meals per day from timed-delivery feeders in their cages. A compound acoustic-visual CS was presented for the 4 minutes preceeding each meal for one group of rats ("signaled", n = 7; see Weingarten, Science, 220,431, 1983 for details), but was completely dissociated from meal delivery in another group of rats ("nonsignaled", n = 5). A third group of rats was never exposed to the CS prior to test, and had ad-libitum rather than intermittent access to food except on the test day when they were food deprived for 6 to 9 hours ("ad-lib", n = 2).

Even though the "signaled" group showed reliable conditioned feeding (6.6±0.5ml with a 2.8±0.8 sec latency), it exhibited no change of plasma insulin and glucose levels from baseline during the 4 minute CS presentation. As compared to the ad-lib group, both the signaled and non-signaled groups showed a higher eating rate and an acceleration of digestive processes as indicated by faster rising glucose and insulin levels.

These results suggest that while feeding behavior is brought under stimulus control in the conditioning paradigm used, the cephalic phase of insulin secretion is not. The control of the acoustic-visual CS appears to be associated with activity or arousal rather than autonomic responses. The question of whether an effective CS for the elaboration of a conditioned insulin response must engage gustatory or olfactory afferents is currently under investigation. (USPHS grant AM27627).

ANGIOTENSIN AND DRINKING

295.1 THE DEPENDENCE OF SALT APPETITE ON A SYNERGY OF ALDOSTERONE AND ANGIOTENSIN. A.N. Epstein, R.R. Sakai*, B. Stamoutsos* & Jacob Schultz*. Dept. of Biology & Institute of Neurological Sciences, Univ. of Pennsylvania, Phila. Pa. 19104.

A salt appetite is aroused in the sodium replete rat by treatment with angiotensin and DOCA the precursor of aldosterone (Fluharty & Epstein, Behav. Neurosci. 97, 1983; Zhang et al, Physiol. Behav., 1984), and can be suppressed in the sodium deplete rat with a drug (captopril, CAP) that blocks the production of endogenous angiotensin II (Moe et al, Amer. J. Physiol., 1984). These findings favor the idea (Epstein, Peptides 3, 1983) that a synergy of aldosterone (ALDO) and angiotensin (ANG), the hormones of sodium conservation, are the cause of the salt appetite that occurs under natural circumstances of sodium deficiency.

We have now shown 1) that the synergy can be produced with ALDO, and 2) that the salt appetite of the adrenalectomized (ADREX) rat is especially sensitive to suppression by CAP.

Rats (N=8) fitted with intracerebroventricular (ICV) cannulae received ALDO (40 µg/day, sc) for five days while they were maintained on Purina pellets, fresh water and 3% saline. There was no increase in 24 hour saline intakes. On the fifth day a pulse ICV injection (pICV) of ANG II (0.6, 6 or 60ng) or saline was given. Water and 3% saline intakes were recorded at 30 mins, 3 and 24 hrs thereafter, as well as the animals' latencies to drink each fluid. The combined treatment with systemic ALDO and pICV ANG increased saline intake relative to either treatment alone. Latency to drink saline was also shorter in the synergy group.

ADREX rats (N=8) that were drinking 15.1 ± 4.5 ml during 2 hrs of daily access to 3% NaCl drank only 1.9 ± 1.9 ml after one day of free access to water adulterated with doses of CAP (1-2 mg/ml) that do not reduce the salt appetite of the non-ADREX Na+ deficient rat. Need-free ADREX rats (salt appetite reversed by therapeutic doses of ALDO) w/o CAP show comparable 3% saline intakes (1.2 ± 0.9 ml) to those of the CAP-treated ADREX rats. This suggests that the suppression by CAP is a complete abolition of the need-induced appetite. Self-administration of systemic CAP had no effect on the water intake of the ADREX rats when it was only available for 2 hr/day.

The salt appetite of adrenalectomy appears to be completely and specifically suppressed by blockade of production of endogenous ANG II.

This work supported by NINCDS 03469.

295.2 CENTRAL AND PERIPHERAL INFUSION OF ANGIOTENSIN-CONVERTING ENZYME INHIBITOR ON SALT PREFERENCE IN SPONTANEOUSLY HYPERTENSIVE RATS. J.Y.H. Chan and J.S. Hutchinson*. Department of Pharmacology, Faculty of Medicine, National University of Singapore, Kent Ridge, Singapore 0511.

Injection of angiotensin II (ANG II) into the cerebral ventricles is known to result in a marked increase in water intake, followed by a sustained ingestion of NaCl solution in the rat. At the same time, the spontaneously hypertensive rat (SHR) possess an exaggerated salt appetite compared to normotensive Wistar-Kyoto controls. Furthermore, centrally produced ANG II, independent of the circulating renin-angiotensin system, is elevated in the SHR. The implicated possibility that such endogenous brain ANG II is involved in the exaggerated salt appetite of the SHR was examined in this study using a two-bottle preference test.

Fluid intake by weight from saline and water bottles was measured daily. The baseline values were established over a 6-day period, after the animals had been allowed to acclimatize for 3 days. To differentiate the central and peripheral effects of ANG II, the angiotensin-converting enzyme inhibitor, captopril, was infused by two different routes, using the Alzet® osmotic minipumps. Captopril was delivered via the intracerebroventricular route to one group of SHR and the intraperitoneal route to another, at a rate of 80 µg/hr. Infusion of saline, at comparable rate and routes, served as vehicle controls in two other groups of animals. The effects of these treatments on fluid intake and preference were followed for 6 days.

During the control period, SHR drank 24.99± 2.98 ml per 100 g body weight per day of saline and 2.12 ± 0.55 ml per 100 g body weight per day of water. This represented a saline preference of 92% of total fluid intake. Central captopril infused rats showed a significant decrease in saline intake during the treatment period compared to the pretreatment period ($F_{1,5} = 67.27$, $P < 0.001$). In contrast, peripheral infusion of captopril did not produce a significant change in saline intake ($F_{1,5} = 1.65$, $P > 0.20$). Vehicle-treated SHR showed no change in saline intake during the peripheral or central captopril infusion period.

In conclusion, the selective attenuation of the exaggerated salt intake of the SHR by centrally applied captopril suggests that elevated central ANG II may contribute to this elevated salt appetite. (Supported by a grant, RP 45/83, from the National University of Singapore.)

295.3 COMPARISON OF ANGIOTENSIN II AND III-INDUCED DIPSOGENICITY AND PRESSOR ACTION. J.W. Wright, S.L. Morseth, M.J. Sullivan* and J.W. Harding. Departments of Psychology and Veterinary Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164.

The primary sites responsible for angiotensin-induced dipsogenicity and pressor action in the laboratory rat appear to be in two forebrain circumventricular organs (CVOs), the Organum vasculosum of the lamina terminalis and the subfornical organ. Our laboratory has recently measured specific [^{125}I]AIII binding in these CVOs taken from rats, gerbils, rabbits, and monkeys suggesting a role for AIII in the central control of drinking and blood pressure control.

In the present investigation we initially compared AII and AIII-induced blood pressure changes via brachial artery (after Haywood et al. Am. J. Physiol. 239:H108-H113, 1980) or femoral vein infusion in rats at doses of 1, 10, 100 and 500 pM/kg/min for a duration of 10 min. Although the two avenues of infusion resulted in equivalent pressor responding, AII was more potent than AIII particularly at the two highest doses. However, intracerebroventricular (icv) injections of AII and AIII at doses of 0.1, 1, 10 and 100 pM/2 µl resulted in approximately equivalent blood pressure elevations in rats. The latter observation agrees with our recent finding that comparable doses of AII and AIII yield nearly equivalent dipsogenic responses when delivered icv (Wright et al., Brain Research 295: 121-126, 1984). Further, AII and AIII infusion via brachial artery catheter at doses of 1, 10, 100 and 500 pM/kg/min for 8 hr resulted in considerably more drinking to AII than to AIII, especially at the two highest doses.

The present investigation also evaluated the efficacy of [Sar^1-Ile^8]AII in reducing these responses. Additional groups of rats were prepared with brachial artery catheters and were pretreated with a 5 min infusion of [Sar^1-Ile^8]AII at a dose of 10 nM/kg/min followed by 5 min durations of angiotensin infusion (100 pM dose) at 10, 30 and 60 min post-[Sar^1-Ile^8]AII treatment. [Sar^1-Ile^8]AII significantly reduced the AII and AIII-induced pressor responses.

These results suggest that: AII and AIII-induced drinking and pressor responses are equivalent when administered icv at low doses; however, AII is more potent when infused via the brachial artery; and the receptor antagonist [Sar^1-Ile^8]AII effects indicate a common receptor site for AII and AIII. (Supported by the American Heart Association)

295.4 ANGIOTENSIN II-INDUCED DRINKING IN RATS IS INHIBITED BY CLONIDINE, m-OCTOPAMINE AND m-SYNEPHRINE VIA BRAIN PRESYNAPTIC α_2-ADRENOCEPTORS. N. Rowland and M.J. Fregly*. Depts. of Psychology & Physiology, Univ. Florida, Gainesville, FL 32611.

Studies from this laboratory have shown that central (intracerebroventricular, IVT) or peripheral administration of clonidine, an α_2-adrenoceptor agonist, can attenuate the drinking response to a variety of dipsogenic stimuli in rats. Further yohimbine and tolazoline, α_2-adrenoceptor antagonists, augment the water intake to peripherally-administered angiotensin II or isoproterenol. The studies reported here establish a dose-inhibition relationship between the dose of clonidine administered (0.5 to 8 µg, IVT) and inhibition of the drinking response to angiotensin II (200 µg/kg, s.c.). The DI_{50} was 1 µg. Yohimbine (300 µg/kg, i.p.) reversed the antidipsogenic effect of centrally-administered clonidine on angiotensin II (200 µg/kg, s.c.)-induced water intake.

The antidipsogenic effects of the o- m- and p-isomers of both octopamine and synephrine, administered IVT in doses of 10 and 20 µg, were also studied. Of the isomers tested, only d,l-m-octopamine and l-m-synephrine (phenylephrine) attenuated the drinking response to angiotensin II (200 µg/kg, s.c.), and in a dose-related manner. Their antidipsogenic effects could be prevented by concurrent administration of yohimbine (300 µg/kg, i.p.). These results suggest that m-octopamine and m-synephrine exert their antidipsogenic effect via α_2-adrenoceptors. These data further support the idea that central presynaptic α_2-adrenoceptors are involved in the mediation of experimentally-induced drinking in rats. Stimulation of these receptors reduces water intake while inhibition of these receptors enhances water intake.

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295.5 NORADRENERGIC (NE) CELL TRANSPLANTS INTO 6-HDA-DENERVATED VENTRAL LAMINA TERMINALIS NUCLEI RESTORE DRINKING RESPONSES TO SYSTEMIC ANGIOTENSIN II (AII). S.I. Bellin, A. McRae-Degueurce, S. Landas* and A.K. Johnson. Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

Attenuated drinking responses to AII challenges, and confined reductions of catecholamine (CA) histofluorescence intensity in discrete AV3V midline nuclei are specific consequences of intraparenchymal 6-hydroxydopamine (6-HDA, 4 ug/2ul) injections into rat OVLT and median preoptic (MnPO) nuclei (Bellin et al, Neurosci. Abstr., p199, 1983; ibid, Fed. Proc. p1070, 1984). Conversely, AII-elicited drinking persists in 6-HDA-treated animals when desmethylimipramine pretreatments selectively preclude depletion of NE in these nuclei. Here, we extend these findings and strengthen the hypothesis that the functional integrity of NE neurons in OVLT and MnPO nuclei subserve the drinking response to AII. All animals drank at least 2.5 ml tap water during a 2 hr prescreening test to AII (1.5 mg/kg, SC). Two weeks following sham or neurochemical lesioning of OVLT and MnPO nuclei with 6-HDA, values from a second AII drinking test were recorded (vehicle controls = 5.2 ± 0.8 ml; n=6: 6-HDA-treated = 0.9 ± 0.7 ml; n=16). Thirteen of the chemically lesioned rats then received dissociated cell suspensions of A6 (n=7) or A1,2 (n=6) tissues which were excised under sterile conditions from 17-day old rat fetuses and transplanted into OVLT and MnPO nuclei. Three non-transplanted, 6-HDA-treated rats served as treatment controls. Five weeks later, a third AII drinking test was given. Treatment control animals continued to demonstrate response deficits to peripheral AII (0 ± 0 ml). However, 5 of 7 A6 transplant subjects (4.9 ± 2.0 ml), and 4 of 6 rats in the A1,2 transplant treatment group (7.5 ± 2.9 ml) drank to prescreening criterion (2.5 ml), with response magnitudes statistically indistinguishable from those observed in vehicle controls (5.6 ± 0.5 ml). Preliminary histological examination of the transplanted loci in cresyl violet-stained sections and by histofluorescence indicate that, in cases where viable transplants of NE cells into OVLT and MnPO nuclei existed, there was a restoration of AII-elicited drinking. Conversely, where the transplants did not appear to survive, AII-stimulated drinking was absent. The new transplant paradigm, employed in conjunction with this unique animal model, provides an opportunity to selectively study neurobiochemical correlates of body fluid balance mechanisms and hypertension pathophysiology which may be associated with specific neurotransmitter deficits or organic dysfunction in discrete brain nuclei.

295.6 ANGIOTENSIN II PRODUCES A TASTE AVERSION IN CATS BUT NOT IN RATS. B. M. Rabin, W. A. Hunt*, A. C. Bakarich*, A. L. Chedester*, and J. Lee*. Behavior. Sci. and Vet. Med. Depts., Armed Forces Radiobiol. Res. Inst., Bethesda, MD 20814, and Dept. Psychol., Univ. Maryland Baltimore County, Baltimore, MD 21228.

Despite the fact that the area postrema (AP) of the rat contains binding sites for Angiotensin II (AII), lesions of the AP in the rat do not eliminate the hypertensive effects of AII injections as do AP lesions in the cat (Simpson, Neuroendocrin., 32: 248, 1981). These results suggest that the AP of the cat is more responsive to exogeneously administered AII than that of the rat. Because previous research (Rabin et al., Radiat. Res., 93: 388, 1983) has established that the AP mediates the acquisition of a conditioned taste aversion (CTA) following exposure to ionizing radiation or treatment with a variety of drugs, these results would suggest that peripheral injection of AII would be more effective in producing a CTA in cats than in rats.

Using a single-bottle CTA procedure, injection of AII (1 mg/kg, i.p.) in cats immediately following ingestion of chocolate milk on the conditioning day caused a significant 80% decrease in milk intake when tested 48 hrs later. Controls, given saline injections on the conditioning day, showed a slight increase in chocolate milk intake on the test day. In contrast, rats given an injection of AII (1 mg/kg, i.p.) following ingestion of a 10% sucrose solution on the conditioning day showed no significant change in test day sucrose intake compared to saline-injected controls. A second experiment examined the effects of AP lesions in the rat on the CTA response to AII injection using the more sensitive two-bottle procedure. With this procedure, AII injection produced a slight, but significant decrease in sucrose preference (although 12 of 15 rats continued to show a preference for the sucrose solution), whereas the rats with AP lesions showed no change in sucrose preference.

These results indicate that there is a relationship between the physiological sensitivity of the AP to AII and the capacity of the hormone to produce a CTA. They also suggest that activation of the AP may be a sufficient condition for the acquisition of a CTA.

296.1 DISTRIBUTION OF NEURONS SENSITIVE TO NEUROCHEMICALS AND ITS FUNCTIONAL INVOLVEMENT IN THE MONKEY AMYGDALA. Y. Oomura, Y. Nakano*, L. Lenard*, S. Aou, T. Yamamoto*, and H. Nishino. (SPON: S. Mori) Dept. of Biological Control, Nat. Inst. Physiol. Sci., Okazaki 444, Japan; Inst. of Physiol., Univ. Med. School, Pecs H-7643, Hungary.

Extracellular neuronal activity was studied in the monkey amygdala and the effects of electrophoretically applied noradrenaline (NA), dopamine (DA), acetylcholine (ACh), morphine (Mo) and glucose (Gluc) were examined. The behavioral paradigm was a high fixed ratio bar press task (FR 20) signaled by a cue light and rewarded by a cue tone. Out of the 186 neurons tested, 68% exhibited changes in the firing pattern during food task. The predominant effect of DA (23%) and ACh (30%) was an increase in the firing rate while that of NA (31%) and Mo (30%) was a decrease. In 15% of the neurons, a reduction in firing rate was observed after Gluc application (Gluc-sensitive neuron). The number of NA sensitive neurons was more in the lateral part of amygdala than in the centromedial part, while DA, ACh and Mo sensitive neurons were found more often in the later. Gluc-sensitive neurons were found only in and around the central nucleus. The distribution of catecholamine sensitive neurons was comparable to the anatomical data. The population of cells which responded to the diffeent phases of the task were almost similar in the centromedial and the lateral part. But inhibitory responses to the different phases of the task was observed more often in the centromedial part than in the lateral part. In general, drug sensitive neurons changed their activity significantly during the task, and a majority of them changed their firing pattern when trials were made with more palatable food (raisin) or no food (extinction). Functional correlations between drug sensitivity and pattern of the task were observed in the centromedial part. The activity of NA, Mo, and Gluc-sensitive neurons significantly decreased during bar press, while that of DA sensitive neurons increased. On the other hand, there were no such correlations in the neurons of the lateral part. These results suggest that amygdalar neurons in the centromedial and lateral part may possibly be related to the food related response but in a different manner.

296.2 Effects of an Environmental Mycotoxin, Zearalenone on Feeding and Body Weight at Estrogen-Sensitive Brain Sites. C. Wayne Simpson* (Spon. Stanley R. Nelson). Dept. of Biology and School of Medicine, University of Missouri-Kansas City, K.C., Mo. 64110

Zearalenone is synthesized in nature by a fungus that infests grains in the field. Zearalenone is a non-steroid resorceryl lactone which in sufficient quantities in vivo has been associated with hyprestrogenism in both livestock and laboratory animals. Although these data imply an alteration of estrogen action almost no studies have investigated the CNS mechanism of action of this mycotoxin. This experiment investigated the effects of zearaleone on feed intakes and body weights of rats when placed directly at estrogen-sensitive brain sites. Rats, under ketamine anesthesia, were implanted with a unilateral stainless steel cannula directed in different groups of animals to estrogen-sensitive sites in the lateral preoptic area, bed nucleus of the stira medullaris, ventromedial nucleus of the hypothalamus, and the perifornical area of the dorsal hypothalamus. Zearalenone, estrogen or cholesterol was implanted at each site in each animal over several weeks. The compounds were placed at the site through a smaller gauge stainless steel injector filled by the tap method. Injectors were filled, weighed and placed in the site for 24 hours. Food intakes and body weights were measured daily for all animals to the nearest 0.1 gm until body weights and food intakes returned to control levels. Table 1 shows the comparison of the mean maximum response on 24 body weights when rats were injected with zearalenone or on different days cholesterol at the four estrogen sensitive brain sites.

Brain Site	Zearalenone	Cholesterol		
LPO	X = 9.48	X = 2.97	*	* P<.05
	S.D. = 9.35	S.D. = 9.32		** P<.02
SM	X - 12.0	X = 3.46	**	*** P<.01
	S.D. = 7.89	S.D. = 14.17		
VMH	X = -23.17	X = 4.78	**	
	S.D. = 20.09	S.D. = 32.58		
PVN	X = -16.74	X = 0.09	***	
	S.D. = 14.15	S.D. = 20.63		

Additional data concerning time course of the effects on food intakes and body weights as well as dose-response curves will be discussed. The data suggests that zearalenone acts similar to estrogen at some sites but distinctly different at others. Zearalenone may prove to be a useful molecular probe to investigate the estrogenic control of feeding and body weight regulation at estrogen-sensitive brain sites.

296.3 OBESITY IN ABSENCE OF FOOD-RESTRICTED HYPERINSULINEMIA IN FEMALE RATS WITH NONIRRITATIVE LESIONS OF THE VENTROMEDIAL HYPOTHALAMUS. B. M. King, K. R. Esquerre*, and L. A. Frohman*. Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148 and Division of Endocrinology and Metabolism, Univ. Of Cincinnati College of Medicine, Cincinnati, OH 45267.

Damage to the ventromedial hypothalamus (VMH) results in hyperphagia and marked obesity in a variety of species including humans. Plasma insulin levels have consistently been found to be elevated during food-restriction or pair-feeding of animals with VMH lesions, leading several investigators to conclude that hypothalamic obesity is the result of hyperinsulinemia. It has recently been reported, however, that obesity-inducing knife cuts between the ventromedial and lateral hypothalamus do not result in hyperinsulinemia unless the animals are allowed to overeat (Sclafani. A., Diabetologia 20: 402-410, 1981). The present experiment examined whether irritative metallic ion deposits which result from standard anodal electrolytic lesions with stainless steel electrodes contribute to hyperinsulinemia.

Plasma insulin and glucose levels were assayed after a 4-hr fast and 17 min after the initiation of a meal (6 ml sweetened milk in 7 min) both during food-restriction and during ad libitum feeding in female rats with irritative, nonirritative (cathodal electrolytic with platinum electrodes), or sham lesions of the VMH. Histological analysis revealed heavy metallic ion deposition at the site of all irritative lesions, but no deposits in 7 of 9 rats with nonirritative lesions. Plasma insulin levels of rats with irritative lesions were significantly elevated compared to sham-operated animals during food restriction (3.0-3.5 g every 4 hr for 8 days) and the hyperinsulinemia was further exacerbated by hyperphagia during unrestricted feeding. The seven rats with successful nonirritative lesions displayed hyperinsulinemia only when allowed to overeat, and then only under the postabsorptive condition. Both groups with lesions became obese during ad libitum feeding, but the mean 20-day weight gain for animals with nonirritative lesions was only 65% of that observed in rats with irritative lesions (114.7 vs. 175.3 g, respectively). It is concluded that VMH hyperphagia is largely the result of tissue destruction while hyperinsulinemia is the result both of irritative metallic ion deposits (accounting for up to 40% of the weight gain in female rats) and pancreatic β-cells made hyperresponsive on the basis of hyperphagia.

296.4 PERFORMANCE VERSUS COMPETENCE DEFICITS IN INSULIN INDUCED FEEDING IN THE DECORTICATE RAT. Jay Schulkin & H.J. Grill, Dept. of Psychology, New York University, N.Y.C. 10003 and the Univ. of Pennsylvania, Phil. Pa. 19104, (sp. Adler).

In brain lesion research it is often difficult to discern whether a behavioral deficit is the result of impairments in the performance of the animal e.g. execution of the response, or whether it is an impairment in the competence of the animal, e.g. to detect and initiate compensatory behavior. In this regard, it has been reported that frontal neocortical lesioned rats are unable to respond to insulin induced hypoglycemia with compensatory feeding (Brandes & Johnson, 1977, P & B). In this study, however, an unusually high dose of insulin was used--32U/kg. This dose may have impaired the animals performance. We simply lowered the dose to a less dibilitating one--4U/kg and used the complete decorticated rat to determine whether the neocortex is essential for the integration of insulin induced feeding. We found that it was not.

Six decorticate and six control rats were used. The decortication was accomplished in either one or two stages by visually guided aspiration of the neocortex. Testing began 6 months following the surgery. Rats were maintained ad libitum with water, and sweetened condensed milk (in 75 mm cans) as their food source. Following adaptation to the milk diet decorticate rats were injected with the insulin, or isotonic saline, in counterbalanced order. Food was removed for an hour following the injection and then returned; intake of milk was then measured for one hour. Both groups treated with the insulin approached the food source and began to feed as soon as they had access to the food. Decorticates ingested significantly more food following the insulin injection, than following the control injection ($p<.05$, 13.0 gm vs. 5.6 gm). Though the decorticates demonstrate the competence to respond to the insulin challenge they nonetheless manifested deficits (but N.S.) in the magnitude of their milk intake when compared to intact rats (20.2 gm vs. 13.0 gm). These deficits may reflect non-specific sensory-motor impairments or performance deficits that resulted from the brain trauma and further aggravated by the acute insulin challenge. The decorticates were ataxic following the insulin injections; they had difficulty maintaining their posture while ingesting the food. It is also known that decorticate rats have oral-motor impairments and other sensory-motor impairments, and acute regulatory challenges can reinstate the severe behavioral deficits that results from brain trauma. These performance deficits may result from brain trauma in general.

296.5 2-DEOXY-D-GLUCOSE (2DG) AND FEEDING: PERIPHERAL OR CENTRAL ACTION? L.L.Bellush, L.Watkins*, J.Carlton and N. Rowland. Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

Glucose antimetabolites such as 2DG elicit feeding inrats and many other species, notable exceptions being hamsters, gerbils and deermice. Intravenous (iv) infusions of sodium DL-β-hydroxybutyrate (NaHB) attenuate feeding in rats after 2DG (Stricker & Rowland, 1978). Since ketones such as HB can be utilized in vivo by brain as an alternate metabolic fuel to glucose, the data were interpreted in terms of a cerebral origin of hunger after 2DG. The present experiments further examine the effects of ketones and 2DG on feeding.

Study 1: Rats received injections of NaHB or sodium acetoacetate (NaAA) (10 mmol/kg, sc) at the start of the dark phase. Food intake in the next hour was suppressed from control levels by NaAA (-4.0g) and NaHB (-2.3g), effects which persisted in magnitude for 6h. Thus both ketones suppress feeding by about 2x the nominal injected energy. These data do not entirely support the puzzling report by Langhans et al. (Phys.Beh. 31:484, '83) that NaHB suppressed spontaneous nocturnal feeding, but that NaAA did not.

Study 2: Rats with indwelling jugular catheters recieved 2DG (200 mg/kg, sc) followed by iv infusions (4.6 ml/2h) of various substances. Food intakes were recorded at 30, 60 and 120 min (only the latter are presented). The mean±SE changes in food intake, from a mean of 4.5g/2h in 2DG-0.15M NaCl control, are shown in the Table. Hypertonic NaCl suppressed intake as much as the corresponding NaAA and NaHB solutions. The non-ionic enantiomers of butane-1,3-diol (BD) were also infused, in this case for an additional 60 min preceding the 2DG. The (R)-BD is converted to HB in vivo, but had a nonsignificant effect on intake; the non-utilized (s) form suppressed intake. In all cases, the Nut infusions produced total plasma ketone levels of about 2mM. We thus conclude that ketones have no specific satiating effects in this paradigm, or in parallel studies with insulin feeding.

iv infusate	Intake(g/2h)
1.1 M NaCl	-1.5±0.7
2.2 M NaCl	-3.5±0.9
1.1 M (S)-BD	-1.7±0.5
1.1 M NaAA (Nut)	-1.6±0.6
2.2 M NaHB (Nut)	-2.2±0.3
1.1 M (R)-BD(Nut)	-0.5±0.8

Note: Nut=utilizable energy

Study 3: Hamsters do not eat in response to peripheral 2DG but did increase their food intake in response to 2DG (5mg) administered into the cerebral ventricles (0.9g/2h compared to 0.5g/2h after saline or glucose injections). Separate central and peripheral mechanism of action of 2DG are indicated by these results. Supported by BNS 82-16528 from NSF.

296.6 ADULT ZINC DEPRIVATION AND CHELATION: ANOREXIA, HYPERREACTIVITY, & 8-ARM MAZE BEHAVIOR. M.D. Chafetz, S. Barbay*, J. Cronin* and J. Duhon*. Psych. Dept., Univ. Louisiana, Lafayette, LA 70504.

The micronutrient model of anorexia (Chafetz, 1984) predicts that an animal will become anorexic if it is prevented from optimizing nutrient intake. Accordingly, dietary exclusion of any essential nutrient should lead to anorexia, as should any treatment that prevented the occurrence of the normal biochemical consequences of nutrient intake. Because high levels of hippocampal zinc permit a focus on a neural system involving an essential nutrient, we sought to test whether dietary or pharmacologic manipulations of zinc might result in a failure to optimize nutrient intake.

Adult zinc deprived (ZD) animals were compared to food restricted controls on several tests of their ability to optimize nutrient intake. The ZD animals were impaired on their ability to forage in an 8-arm radial maze if the food rewards excluded zinc, but only if the primary zinc deprivation occurred as a result of home cage feeding. Animals who obtained their total daily ZD food by foraging for it on the 8-arm maze were not impaired relative to controls. In home cage feeding experiments, ZD animals exhibited anorexia relative to controls and to their own baseline levels. In a separate experiment, ZD animals were compared to pair fed, ad lib, and neocuproine (zinc chelator) injected animals on a battery of behavioral tests. Discriminant analysis revealed that ZD and neocuproine-injected animals were anorexic relative to ad lib controls. Interestingly, both injected and ZD animals were hyperreactive to non-noxious tactile stimuli. Zinc deprivation and neocuproine injection altered dithizone chromagen deposition in the hippocampus. On the basis of these results, we hypothesize that the hippocampus is the primary target for dietary fluctuations in zinc intake.

296.7 INCREASED DOPAMINE METABOLITE LEVELS ASSOCIATED WITH INSULIN REVERSAL OF CANCER ANOREXIA. W.T. Chance, M. Muggia-Sullam*, F.M. van Lammeren* and J.E. Fischer*. Dept. of Surgery, UC Medical Center, Cincinnati, Ohio 45267.

Although insulin (IN) treatment has been reported to stimulate feeding in anorectic tumor-bearing (TB) rats, little information is available concerning associated biochemical changes. Therefore, we investigated the effects of the daily administration of NPH IN (17-20 U/kg, sc) in acute (9-day) Walker (W) 256 and more chronic (35-day) methycholanthrene (MCA) animal models. W 256 tumors were induced in 60-80 g female SD rats, while the MCA sarcomas were transplanted into adult, male, 344 rats. IN treatment was initiated the day after tumor induction in the W 256 study and continued until their sacrifice 7 days later. For the MCA TB animals, IN administration was contingent upon the development of anorexia and continued for 7 days. Saline (SA)- and IN-treated nontumor-bearing (NTB) and SA-treated TB rats served as control groups. All animals were sacrificed 24 hr after the last injection. Concentrations of serotonin (5-HT), dopamine (DA) and norepinephrine (NE), precursors and metabolites were assayed in whole brain in the W 256 study as well as in the hypothalamus (HY), corpus striatum (CS), septal area (S) and amygdaloid tissue (A) in the MCA experiment by HPLC. Although the SA-injected W 256 TB rats exhibited anorexia by day 5, the IN-treated W 256 rats ate normally until sacrifice. IN treatments also restored normal feeding in the MCA TB rats for 5 days. In the W 256 study, significant elevation of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) was observed in both TB groups. In the IN-treated TB rats, the DA metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were increased by 96% and 58%, respectively. In the MCA study, levels of 5-HIAA were increased in both groups of TB rats in each brain area. Although striatal concentrations of DOPAC and HVA were decreased in TB rats, they were normalized by IN treatment. DOPAC and HVA were also elevated in the A of IN-treated TB rats, while HVA concentrations were increased in the S. Levels of NE in the HY and S were elevated in TB rats and were normalized by the IN injections. Therefore, these data suggest that DA release may be reduced in anorectic TB rats and that correcting the anorexia is accompanied by increased neuronal activity of DA neurons. In addition, a more complex interaction of DA, 5-HT and NE neurons is suggested in the mediation of cancer anorexia. Supported by USPHS NCI grant #1 R01 CA36325.

296.8 DIFFERENTIAL JUNK FOOD SELECTION, OBESITY AND LINEAR GROWTH RETARDATION IN MALE AND FEMALE RATS THAT RECEIVED VENTROMEDIAL HYPOTHALAMIC LESIONS SHORTLY AFTER WEANING. L.L. Bernardis, P.J. Davis* and G. McEwen*. Div. Endocrinology, VAMC Buffalo and Dept. Medicine, SUNY at Buffalo, NY 14215.

Male and female Sprague-Dawley rats received bilateral electrolytic lesions in the ventromedial hypothalamic nuclei (VMNL rats). Sham-operated animals served as controls. For 14 days post-operatively the rats were given lab chow and tap water ad libitum whereupon they were fed in addition to lab chow (and tap water) four human junk foods for 42 days: Hostess HoHos, french fries, potato chips and marshmallows. At this time body weight, length (nose-tail) and Lee Index were determined and the experiment terminated. The data were analyzed using a three-way analysis of variance with a polynomial transformation of the time period. Body weights were similar in all groups but VMNL rats were fatter and shorter than controls. Female VMNL rats were fatter and shorter than male VMNL rats. Caloric intake from all foods combined was greater in males than in females and greater in controls than in VMNL rats. Females showed a decrease in caloric intake and males an increase over time. Caloric intake from lab chow was greater in males than in females, with the females showing a decrease over time. Lab chow was the only food that VMNL rats ate more of than controls and french fries was the only food that males ate more of than females. The females also showed a decrease over time. Rats with VMNL ate less french fries, HoHos and potato chips than controls but ate similar amounts of marshmallows. In conclusion, whereas previous studies had shown that male and female VMNL rats fed lab chow attain the same degree of obesity, the present data indicate the availability of junk food together with lab chow, causes VMNL-induced obesity in weanling rats to develop in the presence of lower caloric intake than in controls. The data also show that female VMNL rats become fatter than male VMNL rats and also show greater linear growth retardation. Except for french fries, there is no sex difference in junk food preference. VMNL rats eat less than controls from all junk foods except for marshmallows, of which they eat as much. It appears that VMNL rats eat more lab chow than the more palatable junk foods because the former is nutritionally balanced whereas the latter is nutritionally "empty." This suggests that the weanling VMNL rat has not lost the capacity to select nutritionally balanced foods.

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296.9 STRESS INHIBITS GOLD THIOGLUCOSE LESIONS IN THE VENTROMEDIAL HYPOTHALAMUS. D. F. Brown* and J. M. Viles* (SPON: R. Alper). Combat Casualty Care, Letterman Army Institute of Research, San Francisco, CA 94129; and Department of Zoology, Iowa State University, Ames, IA 50011.

Gold thioglucose (GTG) is a substrate-specific neurotoxin for a group of neural cells in the ventromedial hypothalamus (VMH). Within 24 hours after a single intraperitoneal (IP) injection of GTG, discrete bilateral lesions are detectable in the VMH of mice. This VMH damage produces hyperphagia and obesity. GTG-induced lesion formation in the VMH is dependent on the hormonal status of the mouse. Insulin must be present in the VMH for GTG lesion formation to occur; diabetes abolishes the GTG-induced VMH destruction. Administration of glucocorticoids to a normal mouse prevents GTG-induced necrosis in the VMH. Furthermore, the hyperphagia and obesity associated with GTG-induced VMH destruction is dependent on an intact pituitary-adrenal axis. Since the adrenal cortex apparently plays a major role in the VMH response to GTG, we investigated whether or not stress, a stimulator of glucocorticoid secretion, could alter GTG lesion formation in the mouse VMH. Female CF1 or Swiss Webster mice approximately 3 months of age were used in this study. They were housed at 23°C on a 12-hour light:12-hour dark photoperiod, fed a normal mouse diet, and given tap water freely. Initially food deprivation or cold exposure was utilized to induce stress. After 3 days of starvation or 5 hours of cold exposure (4°C) the mice were challenged with 300 mg/kg GTG IP, and the stress continued until sacrifice. Following routine histology, the VMH was examined at the light microscope level. No necrosis or lesions due to GTG were observed in the VMH, while controls displayed typical VMH damage from GTG administration. In another study mice were stressed with carrageenan (CAR)-induced abdominal irritation. A 0.25 cc bolus of 2.5% aqueous CAR was injected IP and 18 hours later followed by 500 mg/kg GTG IP. Controls received only GTG. Tail vein blood samples were taken immediately before GTG administration and plasma corticosterone (CORT) concentrations measured by radioimmunoassay. Control animals possessed classic GTG-induced VMH damage, while CAR-treated mice showed almost complete inhibition of GTG-induced destruction. CORT levels averaged 37±7 ng/ml plasma for controls and 207±18 ng/ml plasma for CAR-treated mice ($p<0.001$). Our results indicate that the mouse VMH is protected from GTG-induced necrosis by stress and possibly mediated by increased plasma CORT levels.

296.10 CHANGES IN BODY COMPOSITION FOLLOWING AREA POSTREMA/CAUDAL MEDIAL NUCLEUS OF THE SOLITARY TRACT LESIONS IN RATS. T.M. Hyde and R.R. Miselis. Schools of Medicine and Veterinary Medicine and the Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Lesions of the area postrema (AP) and the subjacent caudal medial portion of the nucleus of the solitary tract (cmNTS) cause a syndrome of transient hypophagia and permanent depression of the body weight growth curve in adult male and female rats (Hyde & Miselis, 1983). In the current study, young male Sprague-Dawley rats weighing 120-170 g were housed individually and maintained on a standard pellet diet. Following eight days of baseline measurements, the rats received either AP/cmNTS lesions or sham lesions. The lesions resulted in a transitory period of depressed food intake and a deceleration of body weight gain. Unlike adults, body weight did not drop after lesioning. However, water intake and water/food ratios were chronically elevated postoperatively in the lesioned group for the duration of the experiment. Behavioral verification of the lesion was demonstrated by a heightened intake of Instant Breakfast (Carnation) in a 30 minute test after overnight food deprivation (Edwards and Ritter, 1981). Body composition analysis was performed sixty days after surgery. Lesioned rats weighed 73.4% of controls at time of sacrifice ($p<.005$). Wet organ weights were obtained; liver and kidney weights of lesioned rats were 74.1% of controls ($p<.05$), while epididymal and retroperitoneal fat pads were 57.1% and 50.0% of controls, respectively ($p<.01$). There were no differences in teste weights. Shaved, eviscerated, decapitated carcasses were analyzed for total body water and fat content. There were no differences in water content. However, total body fat content was significantly reduced in lesioned rats (9.0±0.7% for lesions versus 13.2±0.7% for controls, $p<.001$). These studies demonstrate that lesions of the AP/cmNTS region cause a permanent reduction in fat stores. This reduction persists even after the animals return to a normal level of food intake and rate of body weight gain. Further analyses are underway to determine if this is a primary consequence of the lesion or a chronic metabolic change secondary to the postoperative period of transient hypophagia.

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296.11 RESPONSE OF RATS WITH FORNIX TRANSECTION TO VARIATION OF PROCUREMENT COST DURING FORAGING BEHAVIOR. L.A. Flashman* and B. Osborne. Dept. of Psychology, Middlebury College, Middlebury, VT 05753

The purpose of the present study was to explore the effects of a varied procurement cost on the foraging behavior of rats with fornix transection and control rats. An operant analog designed by Collier was used to examine the feeding patterns of the animals under free feeding, low procurement cost (FR5) and high procurement cost (FR80) situations, in an environment with minimal sensory distraction. It was found that animals with fornix transection did not differ from control rats in general consumption total or pattern. The lesioned animals were able to adapt their feeding behavior to the varied procurement cost in the same way that the control animals did. As the procurement cost increased, the number of meals consumed decreased while the meal duration increased. The meal patterns themselves were different for the fornix transected animals and the control group. Lesioned animals eat more meals over the course of a day than do control animals; their meals are of a longer duration, and their intermeal intervals are shorter than those of control animals. During the course of a meal, fornix animals take a larger number of breaks, during which they drink, explore, or engage in activities other than eating. These differences in the feeding pattern were seen across all procurement cost levels. Interpretation of the data seem to support the possibility of hippocampal involvement in behavioral organization sequencing.

296.12 BOMBESIN SUPPRESSES FOOD INTAKE OF AREA-POSTREMA-LESIONED RATS. Francoise Lacour*, Nancy J. Kenney, Jon N. Kott and Stephen C. Woods (SPON: E. Lotter). Department of Psychology, University of Washington, Seattle, WA 98195.

Ablation of the area-postrema and adjacent caudal-medial nucleus of the solitary tract (AP/cmNTS) results in a reduction of food intake and body weight of rats. Food intake of AP/cmNTS-lesioned rats is unaffected by intraperitoneal (ip) administration of cholecystokinin (CCK). This study examines the effect of AP/cmNTS ablation on the reduction of intake induced by another purported satiety-inducing peptide, bombesin (BBS).

The effect of ip administration of 8 ug/kg BBS on food intakes of 15-hr food-deprived AP/cmNTS-lesioned (n=7) and sham-lesioned (n=7) rats was studied beginning 8 days after surgery. Each animal was tested 4 times; twice with BBS and twice with equal volume injections of isotonic saline. The order of injections was counterbalanced. During 1 set of BBS and control tests, rats were fed pelleted laboratory chow, their standard food. For the second set, liquid Ensure diet was presented during the test period. Intakes were measured .5 and 1 hr after ip injection.

Pelleted food: Lesioned rats consumed less than shams of this standard food during the hr following ip saline injection ($F(1,16)=17.9$, $p<.01$). Intakes of sham-lesioned rats were reduced during the first 30-min after BBS administration ($F(1,6)=7.5$, $p<.05$) and remained suppressed at the end of the test session ($F(1,6)=18.6$, $p<.01$). While having no significant effect on intake of lesioned rats during the the first half of the test session, BBS did suppress total 1-hr intakes of these animals ($F(1,6)=7.0$, $p<.05$).

Liquid Ensure: Following saline injection, lesioned and sham-lesioned rats did not differ in test-session intake of Ensure. As was the case with the solid food, 30-min ($F1,6)=20.6$, $p<.01$) and total 1-hr intakes ($F(1,6)=46.3$, $p<.01$) of shams were reduced following BBS administration. BBS treatment also effectively reduced intakes of lesioned rats both during the first 30-min ($F(1,6)=17.8$, $p<.01$) as well as for the entire hour of Ensure access ($F(1,16)=12.5$, $p<.01$).

Thus, BBS reduces food intake of AP/cmNTS-lesioned rats. These data indicate that AP/cmNTS ablation does not eliminate responsiveness to all purported satiety hormones and suggest that the reductions of feeding by CCK and BBS may be independently mediated.

296.13 FURTHER EVALUATION OF MACRONUTRIENT SELECTION OF RATS FOLLOWING AREA-POSTREMA ABLATION. Jon N. Kott and Nancy J. Kenney (SPON: J. Lockard). Department of Psychology, University of Washington, Seattle, WA 98195.

Immediately following ablation of the area postrema and adjacent caudal-medial aspect of the nucleus of the solitary tract (AP/cmNTS), rats decrease food intake and lose weight. For rats maintained in a macronutrient-selection situation, the post-ablation reduction of intake is due exclusively to reduced fat intake. We have recently shown that AP/cmNTS-lesioned rats may develop aversions to foods ingested around the time of lesioning. In this study we examine the possibility that the reduced intakes of fat previously noted for macronutrient-fed lesioned rats is an artifact of the presentation of the diet during the peri-surgery period.

Ten AP/cmNTS-lesioned and 9 sham-lesioned rats were fed pelleted laboratory chow prior to and for the first 25 days after surgery. Separate sources of fat, carbohydrate and protein were then presented for 2 consecutive 10-day periods (Macro 1 and Macro 2). Body weight and total caloric intake were measured daily during the last 7 days prior to macronutrient access (Baseline) and during the period of macronutrient self-selection. Proportions of intake from each of the macronutrient sources were calculated for the selection phase of the study.

Baseline: Daily food intake ($F(1,17)=20.4$, $p<.01$), but not rate of weight gain, of the lesioned rats was reduced compared to that of controls.

Macro 1: As is typical when lesioned rats are offered a new food, total caloric intakes of the lesioned rats increased ($F(1,9)=24.2$, $p<.01$) and did not differ from that of sham-operated controls when the macronutrients were presented. No marked differences in the proportions of calories taken by lesioned and control rats were noted during this transition period.

Macro 2: Total intakes of lesioned rats returned to baseline levels and were lower than those of controls ($F(1,17)=12.0$, $p<.01$). AP/cmNTS-lesioned rats took a smaller proportion of total calories as fat ($F(1,17)=14.7$, $p<.01$) but greater proportions from both carbohydrate ($F(1,17)=7.6$, $p<.05$) and protein ($F(1,17)=4.8$, $p<.05$).

Thus, the reduction of fat intake by macronutrient-fed, AP/cmNTS-lesioned rats appears to be a primary effect of such ablation and is not dependent upon the presentation of the macronutrient diet during the immediate post-surgery period.

296.14 DECREASED NOREPINEPHRINE CONTENT AND TURNOVER IN BROWN ADIPOSE TISSUE FROM SYRIAN HAMSTERS FED HIGH-FAT DIET. J.F. McElroy*, P.M. Mason*, J.M. Hamilton* and G.N. Wade. Div. of Neuroscience and Behavior, Dept. of Psychology, Univ. of Massachusetts, Amherst, MA 01003.

Male Syrian hamsters (Mesocricetus auratus) fed a high-fat (HF) diet can become obese without overeating and exhibit increases in brown adipose tissue (BAT) weight compared to hamsters fed a commercial chow high in carbohydrates. This increased metabolic efficiency is similar to that observed in genetically obese (ob/ob) mice and Zucker (fa/fa) rats. BAT is an important site of adaptive changes in heat production in rodents. Thermogenesis in BAT is controlled by norepinephrine (NE) release at sympathetic nerve endings in the tissue. Changes in BAT sympathetic activity parallel known adaptive changes in heat production. For example, NE turnover in BAT is increased in rats exposed to cold or fed a "cafeteria" diet, whereas NE turnover is reduced in genetically obese mice and rats. The purpose of the present study was to determine whether the HF diet-induced increase in metabolic efficiency is associated with a decrease in sympathetic activity in BAT as reflected by a reduction in NE turnover. NE turnover was determined by the disappearance of NE from the tissue after inhibition of its biosynthesis with alpha-methyl-p-tyrosine.

As previously reported, hamsters fed a HF diet did not overeat, but they tripled their rate of weight gain in a month compared to animals fed a high-carbohydrate diet. Interscapular BAT (IBAT) and heart wet weights were elevated 43 and 9%, respectively, in fat-fed hamsters. High-fat feeding for 30 days decreased NE content and turnover in IBAT by 37% (from 4.4 to 2.8 ng NE/mg tissue) and 75% (from 0.31 to 0.075 ng NE/mg tissue/hr), respectively. NE content and turnover in heart were unaffected by HF diet.

Previous research showed that fat-fed hamsters become obese at least in part because of diet-induced decreases in energy expenditure. In the present study, high efficiency of energy storage following HF diet feeding is paralleled by decreased SNS activity in BAT, a major site of energy expenditure in rodents. Cardiac NE turnover was not altered by fat feeding, demonstrating that the diet effect on NE turnover in BAT is not due to a general reduction in SNS activity. Thus, SNS activity in BAT can be diminished by fat feeding and may be a factor contributing to the enhanced metabolic efficiency seen in these animals. Experiments in progress are evaluating GDP binding and cytochrome oxidase activity in BAT from similarly treated animals.

296.15 FOOD INTAKE AND WEIGHT GAIN OF ZUCKER RATS: INCREASED BY AUTOIMMUNIZATION AGAINST B-ENDORPHIN: C.L. McLaughlin and C.A. Baile, Wash. Univ. Med. School, St. Louis, MO 63110

Opioid peptides are postulated to mediate the hunger component in the control of food intake and regulation of body weight, e.g. increased plasma B-endorphin concentrations are associated with hunger. It was postulated that in rats immunized against B-endorphin the antibodies would sequester B-endorphin resulting in decreased food intake and body weight. Zucker rats have increased B-endorphin concentrations in the brain and neurointermediate pituitary but not anterior pituitary or plasma and were postulated to be more responsive to treatment. Adult Zucker obese (n=20, 568±13g) and lean (n=20, 299±16g) rats were immunized against bovine serum albumin conjugated (treated) or not (control) to B-endorphin. Capacity of serum to bind 125 I-B-endorphin was maximal at 8 wks and was present only in serum from treated obese (721 vs. .3 pmol/l, $p<.004$) and lean (829 vs. 5 pmol/l $p<.001$) rats. During the 12 wk period food intakes were increased in treated obese (31.4 vs. 30.75 g/day, $p<.01$) and lean rats (20.60 vs. 20.32 g/day, $p<.05$) and body weight gains were increased in treated obese (.86 vs. .44 g/day $p<.01$) but not lean rats (.26 vs. .21 g/day, NS). Food intakes and body weight gains were increased more in treated obese than lean rats ($p<.001$ and $p<.02$ respectively. Serum concentrations of free B-endorphin in treated rats were only 10% of those in control obese (8.1 vs. 86 pmol/l, $p<.002$) and lean (19.4 vs. 120 pmol/l $p<.001$) rats. However, concentrations of total B-endorphin (free and that bound to the antibody) were increased in treated obese (119 vs. 56 pmol/l $p<.001$) and lean (133 vs. 97 pmol/l $p<.002$) rats. Weight of the anterior pituitary, likely the source of plasma B-endorphin, was increased in treated obese (14.44 vs. 10.45 mg) and lean (13.38 vs. 10.10 mg) rats ($p<.001$). However, concentrations of B-endorphin in the anterior pituitary were decreased in treated obese (204 vs. 373) and lean (291 vs. 526 ng/mg) rats ($p<.004$) and neither total anterior pituitary content nor hypothalamic concentration of B-endorphin was affected by treatment. Thus in rats autoimmunized against B-endorphin, food intake and body weight gain were increased but it is unclear whether these are responses to the decreased free concentration or increased total concentration of plasma B-endorphin. It is possible that increased production of other proopicortico-tropin cleavage products, e.g. ACTH, in rats with antibodies to B-endorphin may have also contributed to the increased food intake, body weight gain and pituitary size responses found. (Supported by a grant from Monsanto Company).

296.16 RECEPTORS FOR CHOLECYSTOKININ (CCK) AND EFFECT OF CCK ON INDEPENDENT INGESTION IN RAT PUPS. P.H. Robinson*, T.H. Moran*, P.R. McHugh. Johns Hopkins University School of Medicine, Baltimore, MD 21205, Maudsley Hospital, London.

CCK is known to inhibit feeding in a number of species but its site of action may be central or peripheral. Rat brain CCK receptors are found in low concentrations at birth but increase over the first fifteen days of life. Moreover, CCK reduces suckling in 15 day old, but not younger, rats. It has been demonstrated recently that rat pups will show ingestive behaviour when tested in the presence of warm cow's milk, away from the dam.

Young rats, aged 1,3,6,10 and 15 days were given CCK octapeptide, 8mcg/kg ip, or saline vehicle, and then placed on a milk soaked towel in a 35° observation chamber. The amount of milk ingested over 30 minutes was determined by weighing the pups before and after the test. Observers, blind to the treatment, noted the behaviour of the animals during the test period. Other rat pups and foetuses were dissected and their stomachs removed, sectioned and binding sites for CCK were demonstrated by autoradiography.

Mean intake after saline injection was 5% of initial body weight, compared to 2.5% after CCK ($F(1,25)=104.32$, $p<0.001$). Observed ingestive activity (mouthing, licking and gaping) was also reduced by CCK ($F(1,25)=36.03$, $p<0.001$) but there was no significant effect of CCK on non-ingestive motor activity ($F(1,25)=2.2$, $p>.1$).

Specific CCK receptor sites were observed in the pylorus and in gastric mucosa of the 20 day foetus. At 3 days of age specific binding was identified in the circular muscle of the pyloric sphincter and of the pyloric antrum, and in the mucosa of the distal part of the stomach. By 10 days, little mucosal binding was evident and binding was restricted to the muscle of the pyloric sphincter and antrum. In the adult, no antral binding was detectable and CCK receptors appeared to be localized exclusively to pyloric circular muscle.

CCK reduces intake of milk in rat pups aged 1-15 days. That this was a specific effect on ingestion rather than a non specific behavioural effect is suggested by the observed reduction in ingestive but not in motor activity. This behavioural response, combined with the finding that CCK receptors are present in the stomach when brain receptor levels are low, provide evidence for a peripheral site of action for the satiety effect of CCK. (Supported by NIH grant 2-R01-AM19302, Wellcome Trust, London).

296.17 EFFECTS OF NEONATAL AND POSTNATAL ANABOLIC STEROIDS ON FLUID INTAKE IN FEMALE RATS. J. Kucharczyk and J. Lemoine*. Dept. Physiology, Univ. Ottawa, Sch. Med., Ottawa, Canada K1H 8M5.

Dianabol (methandrostenolone) is a synthetic steroid with anabolic actions of longer duration than testosterone propionate (TP) but with fewer androgenic effects when administered to adult animals (Arch. Androl. 6: 83, 1981). We compared the effects of treating female rats with TP and Dianabol on fluid ingestion during the immediate postpubertal period. Female rats injected with 1 mg TP on day 5 after birth weighed significantly more than Dianabol-treated (1 mg) or vehicle-injected control females. There were no differences between groups in 24 h intakes of food or water. TP- and Dianabol-injected rats drank less water than controls in response to acute extracellular dehydration (i.p. polyethylene glycol, 0.5% bwt.), but not after acute cellular dehydration (i.p. 2 M NaCl, 1% bwt.). Neonatal TP and Dianabol treatment was found to significantly increase the volume of the "sexually dimorphic nucleus" of the preoptic area (SDN-PDA). Body fluid-electrolyte balance was also studied in postpubertal female rats injected with Dianabol (150 µg/wk for 6 wks) and subjected to biweekly periods of exercise (40 min medium intensity treadmill running). Rats subjected to exercise plus Dianabol drank significantly more water and excreted greater urine volumes on a daily basis than control animals, but ad libitum intakes of 2.7% NaCl were not different. Animals receiving Dianabol without exercise, or combined Dianabol-exercise treatment, showed an increase in Na retention compared with the pretreatment period. However this effect was significantly greater in the combined steroid-exercise group than in animals given Dianabol alone. Plasma aldosterone levels in the exercised and Dianabol injected-exercised groups were lower than controls during the third and fourth weeks of treatment, suggesting that the Na-retaining action of Dianabol is not due to stimulation of adrenal mineralocorticoids. Thus, in the adult female rat, Dianabol has relatively minor effects on fluid-electrolyte balance unless combined with exercise. In contrast, neonatal Dianabol, like neonatal TP, appears to induce 'male' drinking behavior characteristics in genetic females. This appears to involve permanent organizational changes in the hypothalamic level of regulation.

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296.18 FOREBRAIN LESIONS WHICH RENDER SHEEP ADIPSIC DO NOT ALTER SODIUM APPETITE IN SODIUM DEFICIENCY. J.B. Simpson, D.A. Denton*, R.R. Miselis, M.J.McKinley*, R.G. Park*, E. Tarjan*, and R.S. Weisinger*. Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne Australia.

Radio-frequency lesions of the tissue surrounding the optic recess of the third cerebral ventricle were produced in oophrectomized Merino sheep, each prepared with a chronic unilateral parotid fistula and two indwelling lateral ventricular cannulae. The lesions were centered in the relatively large organum vasculosum laminae terminalis and encompassed adjacent tissue, including the nucleus medianus but not the subfornical organ. Such sheep typically show various hydromineral regulatory deficiencies, including: i)transient to permanent adipsia; ii)failure to drink following systemic or central infusions of hypertonic sodium solutions or of angiotensin II; and, iii)reduced natriuresis and elevated plasma sodium concentrations and osmolality during water deprivation (McKinley, etal.,1984). These sheep, however, show normal maintenance of food intake and body weight if artificially hydrated. We asked if such adipsic animals would show normal sodium appetite during the chronic sodium deficiency produced by saliva loss from the parotid fistula. Animals were trained pre-lesion to bar-press for delivery of 600 mM sodium bicarbonate (2 hr/day access) or water (continuous access) and were fed chaff once daily. Animals appeared to respond appropriately to daily 22 hr sodium loss with operant behavior and ingestion during the daily sodium access period despite their persistent adipsia accompanied by altered plasma sodium and osmolality. Specific experiments examined water and sodium ingestion in several acute experimental situations known to alter water and/or sodium intakes. Intracarotid infusions of hypertonic saline suppressed sodium intake while not increasing water intake; intraventricular infusion of hypertonic Na-CSF likewise decreased sodium intake while not increasing water intake; and 48 hr Na-deprivation increased responding for sodium in normal fashion. These forebrain lesions, which perturb water intake as well as urine formation, apparently did not affect salt appetite seen during sodium deficiency. Salt appetite may then be under different neurological controls than several aspects of water intake and fluid regulation in general.

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296.19 PERSISTENT CHANGES IN SODIUM INTAKE AND PLASMA VASOPRESSIN FOLLOWING ACUTE EXTRACELLULAR FLUID DEPLETIONS. S.P. Frankmann, D.M. Dorsa, & J.B. Simpson, Department of Psychology, University of Washington, and GRECC, VA Medical Center, Seattle, WA 98195.

Acute subcutaneous administration of the colloid polyethylene glycol (PEG) in hyperoncotic concentrations causes temporary isosmotic depletion of the extracellular fluid compartment (ECF). Homeostasis in this state is defended both by renal conversation of water and sodium, and by the ingestion of water and sodium. Following the acute depletion, mean saline intake increased from 5 ml/day to 35 ml/day over a period of 2-3 weeks. Immediately following the acute depletion, mean water intake decreased by 7 ml/day. Both effects persisted for at least 3 months and were not secondary to chronic renal fluid losses. One compensatory mechanism induced by hypovolemia is elevated secretion of vasopressin (AVP). The following experiments examined the relationship between the increased AVP and altered fluid intakes.

Expt. I. Trunk blood was collected from unanesthetized adult male Long-Evans rats at 3 and 5 weeks following an injection of PEG (MW=20,000; 20% w/v; 16.7 ml/kg,sc) or the isotonic saline vehicle. Plasma immunoreactive AVP was elevated post-PEG relative to control. Plasma sodium concentrations and hematocrits were not altered.

Expt. II. Male, Long-Evans (LE), heterozygous (HZ) and homozygous (HO) Brattleboro rats were injected sc with PEG (as above). Intakes of tap water and 0.3 M NaCl were recorded hourly for 8 hrs and then daily for 4 weeks. All rats showed an acute increase in intake of 0.3 M NaCl following the colloid dialysis. However, whereas Brattleboro HZ and LE rats increased saline intake over the 4 weeks subsequent to PEG administration, the Brattleboro HO rats did not show the increased daily saline intake.

Following acute depletion of extracellular fluid induced by hyperoncotic colloid dialysis, there is an increased daily intake of 0.3 M saline. This is accompanied by an elevation in basal immunoreactive AVP levels. Further, the increased daily saline intake may depend upon the elevated plasma AVP levels as is suggested by the consistent low saline intake of Brattleboro HO rats. It is suggested that following acute extracellular body fluid depletion, there are adjustments which act to offset the severity of subsequent depletions, and that among the compensatory alterations is a chronic elevation in plasma AVP concentration.

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297.1 AUTORADIOGRAPHIC TRACING OF CORTICOSPINAL PROJECTIONS IN CATS WITH NEONATAL OR ADULT ABLATION OF ONE CEREBRAL HEMISPHERE. F.E. Gomez, J.R. Villablanca, B.J. Sonnier and M.S. Levine. Mental Retardation Research Center, Depts. Anatomy and Psychiatry, UCLA School of Medicine, Los Angeles, CA 90024.

The motor cortex projections to the cervical spinal cord were examined in cats with adult (N=4) or neonatal (x age 14.7± 8.3 days, N=4) removal of the left half of the telencephalon (hemispherectomy) and in intact controls (N=4). After behavioral studies (companion abstracts), the animals were used for this experiment at a mean post lesion age of 295 ± 126 for the adults and of 555 ± 79 days for the kittens. Starting at 4 mm from the midline, 5-6 injections of tritiated leucine-proline (.2 to .6 µl at 50 µ Ci/UL) were made 1.0 to 1.5 mm in front and behind the right cruciate sulcus at 3 mm depth. Five days later cats were perfused intracardially, frozen coronal spinal cord sections were cut at 50 µ and processed for autoradiography. Sections were developed after a 6 week exposure and injections sites and terminal fields were reconstructed from drawings made under both light and dark field illumination. In intact and adult-lesioned cats the projections were essentially contralateral. The fibers descended in the crossed dorsolateral corticospinal tract and penetrated chiefly in the dorsal horn. At upper levels (Cl - C3) the terminals clustered in the left cuneate n. and in the most medial region of laminae VI, fewer terminals were seen in the medial part of lamina V and dorsal aspects of lamina VII. At lower levels (C7-C8), the terminal fields were less dense but occupied a larger area in similar regions of the same three laminae. In neonatal-lesioned cats the outstanding difference was the presence of terminal fields in the grey matter ipsilateral to the cortical injection. The topography of distribution was similar to that described for the contralateral side but the density of the terminal fields was considerably weaker. These terminals appeared to originate from contralateral fibers which cross the midline. Ongoing computer-assisted densitometry confirms the above observations, but the spinal terminals reorganization appears to be less marked than other anatomical changes which we have hitherto reported for hemispherectomized animals. The absence of ipsilateral terminals in adult-lesioned cats is another example (companion abstract) of a lesser brain reorganization following the adult lesion and this matches the diminished functional recovery of these animals compared to the neonatal-lesioned ones. (USPHS Grants HD-05958 and HD-04612).

297.2 REDUCED REORGANIZATION OF CORTICORUBRAL AND CORTICOTHALAMIC FIBERS IN ADULT VERSUS NEONATALLY HEMISPHERECTOMIZED CATS. B.J. Sonnier, J.R. Villablanca, F. Gómez and J.W. Burgess. Mental Retardation Research Center, Departments Anatomy & Psychiatry, UCLA School Medicine, Los Angeles, CA 90024.

The left half of the telencephalon was removed (hemispherectomy) in 6 adults cats, neurobehavioral studies were performed (companion abstracts) and the animals were used for this experiment (median=282 postlesion days, range= 109-317). Injections (5-6) of tritiated leucine-proline were made 1.0 to 1.5 mm in front and behind the right cruciate sulcus. Cats were sacrificed 5 days later and the brains cut in 50µ coronal sections and processed for autoradiography. Injection sites were reconstructed and computer-assisted analyses of terminal field areas in the red nucleus (RN) were made from 8 A-P sections taken at .5 mm intervals and projected over square grid paper. Total surface area of the RN was computed for each section and the proportional area containing terminals was calculated. Terminal densities were visually estimated using a 0-3 point scale and the number of grid squares containing each density score was also computed for each of the 8 sections. Results: a) only 4 cats had bilateral innervation of the RN and terminal patterns in the left RN (ipsilatal to ablation) were inconsistent; b) the mean proportional area with terminal fields in the left RN was only .15 (.59 for right RN); c) relative terminal densities within that .15 area were 46.65% for minimum (score 1), 26.65% for medium (score 2) and 26.65% for maximum (score 3) densities. In contrast, all our reported neonatally-lesioned cats had consistent bilateral RN innervation and the area with terminals in the left RN was significantly larger (.43, $P<.025$, Scheffé test) although relative terminal density scores were similar. Corticothalamic projections in the present cats were essentially unilateral where in all neonatally-lesioned cats fibers crossed the midline to innervate ventrobasal and intralaminary areas of the left side. The distribution topography of the described crossing fiber terminals was similar to that reported in intact controls although in the latter the pathways were only ipsilateral. Results indicate that there is some CNS reorganization after adult hemispherectomy but that it is much more extensive following a neonatal lesion. The anatomical data fit well with our behavioral results where kitten-lesioned subjects recover more than do comparable adult-lesioned and establish hemispherectomy as an excellent model for studying reorganization-recovery after brain lesions. (USPHS Grants HD-05958 and HD-04612.

297.3 SYNAPTIC REPLACEMENT IN CLARKE'S NUCLEUS AFTER LUMBOSACRAL RHIZOTOMY. M. Murray, W. Battisti, G. Liu, and M. Goldberger. Dept. of Anatomy, Medical College of Pennsylvania and Dept. of Biomorphics, National Defense Medical College, Taipei, Taiwan, Republic of China. (SPON: W. Bridger)

Unilateral lumbosacral dorsal rhizotomy has been shown by light microscopic methods to induce collateral sprouting by intact systems (dorsal roots, interneurons) in the cat spinal cord. In lamina II, quantitative EM showed that dorsal rhizotomy elicits replacement of new synapses contemporaneous with removal of lesioned ones. To determine if rapid synapse replacement is characteristic of spinal cord sprouting we applied the same quantiative EM methods to partially deafferented Clarke's nucleus.

Adult cats were subjected to unilateral lumbosacral (L1-caudal) extradural dorsal rhizotomies which partially denervate Clarke's nucleus. Cats were killed 3-6 days or 3-15 months later and spinal cords were prepared for EM. Sections from L3 segment were trimmed into blocks containing both right and left Clarke's nuclei. Measurements in toluidine blue stained 1µ sections showed no difference in cross sectional area among groups, indicating no shrinkage in nuclear area resulting from the deafferentation. Thin sections containing both nuclei were systematically photographed in the EM. Stereological analysis indicated a decrease in area occupied by axons in the chronically deafferented animals and a marked increase in glial cytoplasm in deafferented nuclei which largely accounts for the maintenance of normal nuclear area in acutely deafferented CN. The area occupied by terminals in acutely deafferented animals is slightly decreased compared to controls but in chronically deafferented animals no difference is seen. In controls, most terminals are axodendritic and contain spherical synaptic vesicles. In deafferented nuclei, slightly more terminals appear to be axodendritic, perhaps because of slight perikaryal atrophy. In acutely deafferented animals the total number of terminals showed a decrease but there was a return to normal numbers in chronically deafferented animals. The rate of replacement of terminals in Clarke's nucleus thus may be slightly slower than that in lamina II. We conclude, however, that in Clarke's nucleus, as in lamina II, partial deafferentation by dorsal rhizotomy induces reactive reinnervation which largely replaces the synaptic contacts lost.

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297.4 **LESION-INDUCED SYNAPTOGENESIS IN HIPPOCAMPUS: CHANGES IN TOTAL SIALOGANGLIOSIDES AND GANGLIOSIDE SPECIES.** S.T. DeKosky, M. Skaggs*, K.J. Anderson*, and S.W. Scheff. Depts. of Neurology and Anatomy, Lexington V.A. and Univ. Kentucky Medical Centers, Lexington, KY 40536.

Transection of the fimbria-fornix disrupts the afferent inputs to the hippocampal formation resulting in massive denervation in this CNS structure. Following such a lesion a well-defined temporal sequence ensues: degenerative debris is removed, followed by axonal sprouting and restoration of the synaptic density to control levels. Because total sialoganglioside is selectively enriched in neuronal membranes and is regarded as a quantitative index of synaptic density and neuronal membrane mass, we assessed total sialoganglisides in the hippocampi of Fischer 344 rats 4, 10, 15, and 30 days following surgical transection of the fimbria-fornix (n = 3-5 animals at each time point). In these same samples, the major ganglioside species (GM1, GD1A, GD1B, GT1B, AND GQ1) were quantitated utilizing high-performance thin layer chromatography. Levels of total lipid-bound sialic acid remained stable 4 days post lesion (DPL), had declined to 75% of controls 10 DPL, and at 15 DPL were 60% of controls ($p<0.01$). By 30 DPL, gangliosides per wet weight had returned to control levels in the denervated hippocampi (increase in gangliosides between 15 and 30 DPL $p< 0.01$). Significant alterations also occured in the individual ganglioside species, with diffuse declines in all moieties (except GT1B) occuring immediately. Levels of GT1B were lower than controls at 4, 10, and 15 DPL, but the decline did not reach statistical significance. By 30 DPL, all species had returned to control percentages except GT1B, which was significantly elevated above controls, despite the return of total ganglioside to normal. The fall, then increase, in total ganglioside parallel the changes in synapse density over time as assessed by electron microscopic analysis, and indicate that dynamic alterations in both total and individual ganglioside species accompany the histological alterations of the post-lesion synaptogenesis process. (Supported by the V.A. Research Service and NIH grants NS00444 and NS16981.)

297.5 DEVELOPMENT OF A NOVEL PROJECTION TO THE AVIAN COCHLEAR NUCLEUS FOLLOWING OTOCYST REMOVAL. H. Jackson and T.N. Parks. Dept. of Anatomy, Univ. of Utah School of Medicine, Salt Lake City, UT 84132.

We have recently found that early otocyst removal in chick embryos, which prevents formation of the cochlear nerve, results in formation of an anomalous projection to the cochlear nucleus (nuc. magnocellularis, NM) from the contralateral NM. This projection develops following both uni- and bilateral otocyst removal, indicating that intact input from the periphery is not required for the formation or maintenence of collateral projections to the contralateral NM. Further, these synapses are apparently functional in the case of both uni- and bilateral otocyst removal subjects in that direct stimulation of NM is capable of evoking unit responses from the contralateral NM.

A key question in assessing the nature of this projection is whether it results from the maintenance of an early, normally transient projection. The alternative, that it is a de novo projection existing only under experimental conditions, is of course impossible to prove. However, examination of a large number of older embryos and hatchlings has convinced us that NM-to-NM projections do not normally exist at stages subsequent to formation of normal cochlear nerve-NM synapses (10-13 days of incubation). We have now extended our study to several 7-9 day-old (during which time NM's projections to its normal target are forming) embryos with unilateral otocyst removal and have found no evidence of anomalous collaterals to NM on the unoperated side. This is despite successful labelling of normal projections originating from NM on the operated side. In these same animals, several examples of anomalous collaterals from the normal NM to NM on the operated side were evident. All available evidence, therefore, indicates that this projection arises de novo subsequent to otocyst removal.

The finding that anomalous collaterals are present prior to the time of normal synapse formation in NM was surprising. It suggests that the signal leading to growth of those collaterals is not related to the failure of cochlear nerve-NM synaptogenesis or the absence of those synapses. This in turn may indicate that the signal initiating sprouting in this case is different from that at the neuromuscular junction and most other CNS sites where sprouting has been demonstrated.

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297.6 SPROUTING OF IPSILATERAL PROJECTIONS TO THE MEDIAL TERMINAL NUCLEUS IN THE OPTIC SYSTEM OF THE ALBINO RAT. C.L. SHEN. Dept. of Anatomy, Col. of Med., Nat'l Cheng Kung Univ. Tainan, Taiwan, Rep. of China.

In a previous study (Baisden,R.H., and Shen, C.L., Exp Neurol., 61:549-560, 1978), we found the existence of an ipsilateral projection into the medial terminal nucleus of the accessory optic system in the albino rat. It also showed that retinal fibers of the young rats are capable of sprouting in response to one eye denervation. The present study is devoted to investigate the effect on sprouting of the remaining eye in mature rat enucleated neonatally and as adults.

Albino rats were used in this experiment. One eye of rat pups (group I) was removed at the day of birth. One eye of group II rats was enucleated at the age of 18 months. Four months later, the rats of group I, group II, group III (one adult rat with one eye congenital blindness) and control group were anesthetized with ether and one eye or the remaining eye was injected with a mixture of ^{3}H-proline and ^{3}H-fucose. Two days postinjection, the animals were sacrified. The brains were removed and processed for autoradiographic visualization of the labeled retinal projection in the accessory optic fiber system. Analysis of the ipsilateral projections indicated an accumulation of silver grains over the medial terminal nucleus of the accessory optic system in all experimental rats but not found in the control one. No ipsilateral projection to the other nuclei in this accessory optic system was found. The silver grain density in the medial terminal nucleus was found heaviest in the congenital one eye blindness rat. It was labeled moderately in the rat enucleated at the day of birth. The ipsilateral medial terminal nucleus of the rat enucleated in adult was labeled lightly but visibly. This study supports the sprouting of the remaining retinal fibers to the ipsilateral medial terminal nucleus of the accessory optic system in rat. However, by increasing the age of rat, it decreases the ability of sprouting into the medial terminal nucleus after one eye enucleation. (Supported by the Nat'l Sci. Counc. of Rep. of China NSC70-0412-B010-30.)

297.7 SPROUTING BY INTACT MOLLUSCAN NEURONS IN VIVO. A.G.M. Bulloch, Dept. Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Neurons of the adult nervous system of Helisoma exhibit a variety of forms of plasticity in response to axotomy. The purpose of the present study was to examine the ability of intact (i.e., non-axotomized neurons) to sprout in vivo in response to animal stress.

The morphology of an identified neuron (buccal neuron 5) was examined subsequent to animal stress by either: (1) bleeding, (2) estivation or (3) exposure to extreme temperatures. Neuron 5 sprouted in response to (1) and (2), but not to (3). The occurrence of this sprout peaked 3 days after animal stress (being present in 50% of preparations) and decreased to statistically insignificant level thereafter.

The neuritic sprout evoked from neuron 5 by animal stress had a characteristic morphology which resulted in its penetration of the dendritic arbor of the contralateral neuron 5. In no case was there evidence that the stress conditions had caused axotomy of any of the neuron 5 processes. This is the first demonstration of sprouting and retraction of a neurite in the undamaged adult nervous system of Helisoma.

The preceding observations suggest the hypothesis that stress involving loss of body fluids causes release of growth factors. The isolated Helisoma nervous system is known to release growth factors which can condition defined medium and promote growth of dissociated neurons. Nervous systems from previously stressed animals were examined for release of growth factors. In accordance with the hypothesis, the ability of the Helisoma nervous system to condition medium was drastically reduced by prior animal stress. Specifically, the extent of neurite outgrowth by dissociated Helisoma neurons was reduced by more than tenfold in medium conditioned by nervous systems from stressed animals ($P<0.01$). Thus, stress appears to have caused the prior release of growth factors.

The above results suggest the interesting possibility that sprouting by intact neurons in vivo and by dissociated neurons in cell culture may be regulated by common trophic factors. Such factors may be fundamental to the plastic capabilities of the adult nervous system. (Supported by MRC, Canada, and Alberta Heritage Foundation for Medical Research; technical assistance by E. Kau).

297.8 **DENDRITIC INJURY EVOKES DENDRITIC SPROUTING ONLY IN AXOTOMIZED LAMPREY CENTRAL NEURONS.** **G.E. Hall* and M.I. Cohen. Biology Department, Yale University, New Haven, CT.**

The distance of the site of axotomy from the soma has been found to influence the site of neuritic sprouting in several invertebrate systems, including cultured Aplysia ganglia. Helisoma ganglia and cricket interneurons. Similarly, 'close' axotomy (within 500 μm) of a group of vertebrate interneurons, the anterior bulbar cells (ABC's) in the lamprey, resulted in extensive neuritic sprouting from the dendrites (Hall and Cohen 83), while 'distant' axotomy at sites 1 cm or more from the soma resulted only in axonal sprouting.

We report here that significant neuritic sprouting from the dendrites of lamprey ABC's can be evoked by surgical amputation of the distal lateral dendrites (dendrotomy) in the hindbrain following a distant axotomy of these cells in the spinal cord. Distant axotomy alone or dendrotomy alone produced little or no sprouting in the dendrites. All cells were examined in whole mount between 1 and 2 months after distant axotomy (2-4 weeks post dendrotomy) following intracellular injection of Lucifer Yellow. Processes were considered sprouts if they (1) extended beyond the normal limits of the dendritic tree, (2) took highly aberrant paths within the normal dendritic field, or (3) had swollen tips. Most processes identified as sprouts met 2 or more of the above conditions.

Dendritic sprouts resulting from combined dendrotomy and distant axotomy were linear and unbranched, often running parallel to the dendritic lesion along the rostrocaudal axis. Most sprouts originated on the lateral dendrite at or near the site of dendrotomy. This pattern differs somewhat from the dendritic sprouting seen following close axotomy, in which sprouting has been observed to occur from all parts of the dendritic tree and the sprouts often followed winding paths.

These results are consistent with a hypothesis where (1) axotomy is required in order for sprouting to occur anywhere in the cell, and (2) injury to one part of the neuron permits sprouting to occur nearby. This could account for the observations that: (a) distant axotomy results in sprouting along the axon near the cut tip, (b) close axotomy causes dendritic sprouting, while (c) dendrotomy alone fails to evoke sprouting. (d) Distant axotomy combined with dendrotomy, however, results in sprouting from both sites of injury in the dendrites and the axon stump. (Supported by NIH Spinal Trauma Grant NS 10174-12).

297.9 PROTEIN PRODUCTION IN THE DENERVATED NEUROPIL OF THE DENTATE GYRUS DURING LESION-INDUCED SYNAPTOGENESIS. L.L. Phillips, R.A. Ogle*, and O. Steward. Dept. of Neurosurg., Univ. of Va. Sch. Med., Charlottesville, VA 22908.

The reinnervation of the dentate gyrus following removal of its normal input from the entorhinal cortex is accompanied by increases in incorporation of protein precursors; these occur specifically within the lamina containing denervated dendrites of the granule cells (Fass, B., & Steward, O. Neurosci. 9: 653-644, 1983). This increase in incorporation is paralleled by increases in the number of polyribosomes associated with dendritic spines (Steward, O., J. Neurosci. 3: 177-188, 1983). Because the increases in incorporation might represent an important aspect of the reinnervation process, we were interested in defining which proteins were synthesized to a greater extent during this period.

Adult male albino rats received unilateral lesions of the entorhinal cortex and survived for 8 d (the time at which protein precursor incorporation in the denervated zone is elevated). Eight to 10 living hippocampal slices were equilibrated for 1-2 hrs in modified Eagles medium, and then incubated in medium supplemented with 3H-leucine (54 Ci/mM;0.3 mCi/ml) for 30 mins. After a 30 min chase with cold medium, the molecular layer was dissected free and its proteins were analyzed with one-dimensional SDS-PAGE. The labelled proteins of whole slices were also evaluated for comparison. Leucine incorporation into protein was examined with fluorography, and selected gel tracks were sliced for quantification of radioactivity. Counts in individual bands were normalized to the total number of counts in the lane and the percent difference between denervated and control sides was determined.

The qualitative pattern of labelling in the dissected molecular layer was consistently simpler than in the whole slices, suggesting that a select group of proteins is produced in the dendritic lamina. Analysis of proteins from the molecular layers revealed increases in labelling of bands at 200kd and 43kd and decreased labelling in a band at 53kd. Since changes were observed in specific bands, the increased incorporation in the denervated neuropil does not indicate a generalized increase in protein synthesis. The MWs of the proteins which exhibit increased labelling are similar to HMW cytoskeletal associated proteins and actin. If such identification is verified, this would suggest local synthesis of cytoskeleton during the dendritic reconstruction accompanying reinnervation. Supported by NIH grant NS-12333 to O.S.

297.10 IS THERE COMPETITION FOR TARGET TISSUE BETWEEN TRANSPLANTED AND INTRINSIC 5-HT NEURONS? F.C. Zhou, and E.C. Azmitia. Department of Biology, New York University, New York, NY 10003.

Microinjection of minced fetal raphe tissue (E 14-16) produce a 5-HT hyperinnervation of the dorsal hippocampus (DHipp) of adult intact rats in one month. 5-HT-immunoreactive fibers are seen in abnormally high numbers in the molecular layer of the dentate gyrus, especially, in the infra-granular zone (Azmitia et al., 1981). However, will this local fetal 5-HT hyperinnervation from transplant raphe substitute or suppress the normal (intrinsic) 5-HT innervation from the distant midbrain raphe nuclei. To answer this question, the anatomical relationship between transplant raphe neurons and intrinsic raphe neurons projecting to hippocampus was studied.

A group of rats received fetal raphe, or hippocampus transplant (E 14-16) into the right DHipp. The tissue was minced and injected in 1-2 ul of a balanced salt solution. Subsequently, a microinjection of 100 nl of 10% HRP was made into ipsilateral DHipp (coordinates at 90°: 4.5 mm anterior, 1.5 mm lateral and 4.5 mm below Lambda suture) of the normal and the transplanted rats a month later. The labeled cells in the median raphe nucleus (MRN) and in the transplant tissue in all groups were observed and counted.

In control group, 295 ± 23 (n = 4) labeled neurons in MRN innervating DHipp were counted (also see Zhou & Azmitia, 1983). A month after fetal raphe tissue was transplanted into the DHipp, 305 ± 27 neurons (n = 4) in MRN were observed innervating DHipp, and 287 ± 18 neurons (n = 3) were observed after fetal hippocampal tissue transplanted into DHipp.
There is no significant anatomical change in intrinsic 5-HT afferent from MRN to the DHipp after raphe transplant within one month. Study of long term transplant in relation to the intrinsic innervation is in progress.

Furthermore, analysis of HRP labeled cells in the raphe transplant was performed in the fetal raphe tissue. Many labeled neurons were seen. This finding is in agreement with previous studies showing a hyperinnervation of adult hippocampus by fetal raphe. However, fewer labeled cells were seen in the fetal hippocampal transplant. This suggests a sparse innervation of the adult hippocampus by fetal hippocampus. Future studies are planned to compare the growth condition between the fetal homologous transplant versus the non-homologous afferent transplant. NSF-Grant BNS-83-04704.

297.11 AXON SPROUTING AND NERVE BRANCHING DURING HINDLIMB PLEXUS FORMATION IN THE AXOLOTL.
J.M. Freeman* and D.P. Davey. Dept. of Physiology, Univ. of Sydney, NSW 2006, Australia.

The axolotl *(Ambystoma mexicanum)* hindlimb first appears as a bud when the body length approaches 20mm. However, as early as 15mm body length, limb segmental nerves (SN) contain approximately 30% more axons than non-limb nerves. The axons within SN16 and SN17, the major contributors to hindlimb innervation, were counted in electronmicrographs of horizontal sections taken at various distances from the spinal cord of a 15mm animal.

The proximal end of SN17 contained 105 axons; 50μm from the distal extremity of the myotomes, the number had dropped slightly to 95 (axon diameter 0.59±0.37μm). By a further 40μm more distal, the number had increased to 155. Within the next 10μm, SN17 bifurcated with one branch diverging toward SN16. Each branch contained approximately 90 axons. The mean axon diameter within the two branches differed significantly: 0.30±0.17μm and 0.52±0.29μm, the rostral branch containing the smaller axons.

SN16 contained 116 axons at the distal extremity of the myotomes, where a small branch of 30 axons diverged rostrally toward SN17. Beyond the fork the main branch contained 120 axons.

The observed increase in the total number of axons beyond the bifurcation of SN17 could result from: (1) single axons forming branches that remain within the same nerve branch; or (2) single axons forming branches with one in each nerve branch. Since no axon branching was observed other than at the bifurcation, and since the number of branches that must have occurred approximates the number of axons, the second of these possibilities seems the most likely. The smaller mean diameter of the axons forming the rostral branch to SN17 compared to either SN17 or the caudal branch, may indicate that these axons sprouted from axons that already existed in the other branch. The invariance of the axons numbers before and after branching in SN16 also suggests that sprouts form the branch.

Such sprouting may serve as a mechanism for ensuring that an individual axon makes contact with its appropriate target region; the inappropriate branch may withdraw. A similar phenomenon has been described for SN9 of *Xenopus* by Prestige and Wilson (*J. Comp. Neurol.* 194:235, 1980). The sprouting there was seen to disappear by later stages.

297.12 THE FINE STRUCTURE OF THE ENDPLATE FOLLOWING LIMB IMMOBILIZATION. B.R. Pachter and A. Eberstein*. Dept. of Rehab. Med., New York Univ. Med. Ctr., New York, NY 10016.

Limb immobilization has often been cited as a model to produce disuse of muscle. The interesting feature of this experimental model is that the continuity of the neuromuscular junctional apparatus is not interrupted. While the experimental literature is replete with studies on the muscle in this condition, there are no reported studies on the fine structure of the endplate region following immobilization induced by casting in rat plantaris muscle.

Female Wistar rats (200-250 g.) had their right limb immobilized in the shortened position. After 21 days of immobilization, the plantaris muscle was removed whole and prepared for electron microscopic examination. The innervation zone of the muscle was localized and ultrathin sections were taken. Every endplate encountered was photographed and examined. Various alterations were found in the immobilized endplates. The most susceptible endplates were found on the type II fiber populations. Both degenerative as well as regenerative changes were observed. The degenerative changes were as follows; the postjunctional folds appeared highly-irregular shaped and attenuated, many nerve terminals appeared highly disrupted, large expanses of postjunctional folds were seen with no overlying nerve terminals, the subjunctional sarcoplasm contained pycnotic soleplate nuclei and sarcoplasmic masses. Evidence of reinnervation (terminal sprouting) was also seen and consisted of an increase in the number of small nerve terminals overlying large expanses of junctional folds as well as the presence of multiple nerve terminal branches occurring within the same primary cleft isolated from one another by Schwann cell cytoplasm. Such ultrastructural evidence of degeneration and regeneration often occurred simultaneously within individual endplates. In conclusion, it would appear that limb immobilization leads to an ultrastructural remodelling of the neuromuscular junction in response to hypoactivity. (Supported by NIHR Grant G008300071).

297.13 MOTOR NERVE SPROUTING: EFFECTS OF α-BUNGAROTOXIN AND ANTI-ACHR ANTIBODY. A. Pestronk, and D.B. Drachman, Dept. Neurology, Johns Hopkins School of Medicine, Baltimore, MD.21205

Motor nerves undergo extensive terminal sprouting, resulting in an enlarged area of neuromuscular contact, when the muscle cells they supply are "functionally denervated." In this study we have investigated the role of acetylcholine receptors (AChRs) newly appearing in such muscles in promoting nerve terminal outgrowth. Terminal sprouting was evoked by functional denervation of muscles induced by presynaptic neuromuscular blockade with botulinum toxin. The amount of sprouting was measured morphometrically in cholinesterase-silver stained neuromuscular junctions.

Our results show that nerve terminal sprouting is inhibited by agents that bind to AChRs. Several parameters of terminal sprouting, including terminal branching and endplate length, were reduced by 1) α-Bungarotoxin, 2) anti-AChR antibody from a patient with myasthenia gravis (MG), and 3) anti-AChR antibody from rats with experimental autoimmune MG. Other types of motor nerve outgrowth such as nerve regeneration after crush were unaffected by these agents. Our results suggest that extrajunctional AChRs in skeletal muscle are one of several factors that play an important role in the control of nerve terminal sprouting.

297.14 ANTIBODIES TO NERVE GROWTH FACTOR INCREASES SENSORY AXON NUMBERS IN VIVO. C.E. Hulsebosch, J.R. Perez-Polo+ and R.E. Coggeshall. Marine Biomed. Inst., +Human Biolog. Chem. and Genetics, Univ. of Tex. Med. Branch, Galveston, TX 77550.

We previously found a 10-15% increase in unmyelinated dorsal root axons ipsilateral to spinal denervation in response to hemisection or to unilateral section of neighboring dorsal roots. This increase was interpreted as sprouting. To manipulate the sprouting, nerve growth factor (NGF) was 1) presented in excess or 2) removed in the above paradigms. The excess NGF had no noticeable effect. By contrast, removal by administration of antibodies to NGF (3 µl whole rabbit sera/gm.) in hemisected animals increased the number of unmyelinated dorsal root axons by approximately 7% on the operated side and 50% on the unoperated side.

The present study is designed to pursue these findings by determining the effects of removal of NGF in neonatal rats with no surgical denervations. Shown below are unmyelinated axon counts from the fifth thoracic dorsal roots from 1 month old rats treated with ANTI-NGF from birth as compared to untreated littermates.

RAT #	ANTI-NGF		RAT #	UNTREATED	
	LF	RT		LF	RT
1	6053	5622	5	4276	3574
2	5343	6498	6	3364	4643
3	6868	5979	7	4135	4295
4	6073	5116	8	4066	4423

These figures show a statistically significant increase in unmyelinated axons in the ANTI-NGF rats ($p < .0001$). Although the precise mechanism for the increase is not clear, the data indicate that endogenous NGF has an effect on the number of dorsal root axons. A hypothesis is that interruption of the availability of NGF to dorsal root axons could be interpreted by dorsal root ganglion cells as the chemical correlate of denervation.

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297.15 GM_1 GANGLIOSIDE ADMINISTRATION DOES NOT INDUCE SPROUTING IN DOPAMINERGIC NIGROSTRIATAL NEURONS FOLLOWING A UNILATERAL ELECTROLYTIC SUBSTANTIA NIGRA LESION. I.J. Dunkel*, L.S. Jones and J.N. Davis. Neur. Res. Lab., VA Med. Ctr., Durham, NC 27705 and Depts. of Med. (Neur.) and Pharm., Duke Univ., Durham, NC 27710.

Sprouting is where uninjured neurons expand their terminals to replace damaged neurons projecting to the same target. We chose to study sprouting in the nigrostriatal system because we wanted an adult animal model where the anatomy and neurotransmitter had been well characterized. This system was thought not to sprout in response to a lesion, but Toffano et al. (Brain Res., 261:163-166) found that GM_1 stimulated striatal tyrosine hydroxylase (TH) recovery following hemitransection. We decided to explore the effect of GM_1 on electrolytic lesions that spare the contralateral projection.

Male albino rats were electrolytically lesioned in the right substantia nigra, pars compacta. Animals treated with GM_1 received 30 mg/kg i.p. daily, from the second day post-lesion up to the day before sacrifice. Animals were sacrificed at designated time periods and striatal dopamine levels were measured by alumina extraction and HPLC determination of catecholamines. Following sacrifice, lesions were examined histologically and mapped. Controls included sham lesions, untreated lesioned animals (*), and lesioned animals treated with saline (SAL) i.p.

Dopamine depletion correlated well with the extent of the lesion. The results are expressed as the ratio of right/left (lesioned/unlesioned) striatal dopamine:

2 day *	9-17 day *	14 day SAL	14 day GM_1	21 day GM_1
.42 ± .10 (N=9)	.46 ± .09 (N=9)	.48 ± .13 (N=5)	.21 ± .10 (N=5)	.20 ± .12 (N=5)

These results indicate that GM_1 does not induce the recovery of striatal dopamine expected in a sprouting system following an electrolytic lesion that spares the contralateral projection. This finding contrasts with the work of Toffano et al., and may reflect a differential effect of GM_1 on recovery following neuronal destruction versus axotomy. Alternatively, GM_1 may stimulate TH activity without increasing striatal dopamine.

(Supported by NS-06233.)

297.16 GANGLIOSIDE ENHANCEMENT OF NEURONAL REPAIR MECHANISMS. F. Di Gregorio*, D. Janigro*, F. Vyskocil*° and A. Gorio. *Fidia Research Laboratories, Dept. of Cytopharmacology, 35031 Abano Terme, Italy. °Institut of Physiology, Czechoslovak Academy of Science, Prague, Czechoslovakia.

Our laboratory and, more recently, others showed that Gangliosides (GA) enhance sprouting of regenerating neurons in vivo and in vitro, and inhibit neuronal retrograde degeneration. In addition we found that GA improved the neurological deficits of diabetic neuropathy. All these effects could be ascribed to membrane or metabolic changes induced by GA, which we investigated using neuromuscular preparations and hippocampal slices in vitro. Neuromuscular preparations were incubated with K^+-free solutions for several hours, the resting membrane potential (RMP) decreased to about -60 mV for both 2 hour GA treated and controls. The readmission of K^+ caused a transient RMP hyperpolarization, which was 35% higher in GA treated muscles indicating that membrane pump electrogenic activity was increased by GA. However, if neuromuscular preparations were incubated for 6 hours in GA prior to K^+ readmission or mice were treated in vivo with 10 mg/kg of GA for 3 days, Na-loading was not observed. Such protective effects on membrane potential and m.e.p.p. frequency were also observed under hypoxic conditions, when the nerve-muscle preparations were maintained for 2 hours in O_2-free conditions. Transverse hippocampal slices 500 um thick were perfused with ACSF bubbled with 95% O_2 + 5% CO_2. Hypoxia was induced by decreasing O_2 to 14%. Recordings were taken from CA3 pyramidal cells, stimulated with a bipolar electrode placed in the mossy fiber region. Hypoxia induced a depolarization of 48.7 mV (± 5.7 s.e.) which led to a complete unexcitability of the preparation. The latency to the depolarization was 2,75 min (± 0.4). Readmission of O_2 induced a rapid recovery and a large transient hyperpolarization. Addition of GM_1 (5×10^{-7}M) seemed to be effective in increasing the latency to the depolarization (6.9 ± 1.96 min) and in reducing the depolarizing and hyperpolarizing response (31.8 ± 4.5 mV and 15 ± 2.5 mV respectively). These data are indicating that gangliosides activate membrane processes such as ionic pumps; however in a second stage these molecules show a strong protective action which may indicate a metabolic shift of the affected neurons. We are now investigating whether one of the two phenomena or both are responsible of GA action on neuronal survival and sprouting.

297.17 GONADAL STEROIDS INFLUENCE AXONAL SPROUTING IN THE HIPPOCAMPAL DENTATE GYRUS. J.K.MORSE, S.W.SCHEFF, and S.T.DEKOSKY. Depts of Anatomy and Neurology, Univ. of Kentucky and V.A. Medical Center, Lexington, KY 40536.

Plasticity in the CNS has been studied using the hippocampal dentate gyrus as the model. Following a unilateral lesion of the entorhinal cortex, a deafferentation of the granule cell dendritic tree occurs, along with a growth of residual fibers. This morphological change involves the growth of the commissural and associational fibers innervating the dentate molecular layer. The influence of estrogen (E) on this fiber plexus growth, has been previously reported by us. The present experiment was designed to extend these findings and to evaluate the influence of testosterone (T) on the reactive growth fibers.

Young adult animals of both sexes were randomly assigned to one of 6 groups: 1)Normal untreated, 2) Normal with E implant (serum concentration of 75pg/ml), 3) Normal with T implant (serum concentration of 2.5ng/ml), 4) Castration untreated, 5) Castration with E, and 6) Castration with T.

All animals were subjected to a sham or complete castration followed 7 days later by removal of entorhinal cortex and implantation of hormone capsules. Following a 15 day survival period, animals were killed and their brains examined for changes in hippocampal morphology using the Holmes Fiber stain or HRP.

Castrated female animals (group4) show a significant decrease in sprouting when compared to female controls (group 1) or females with E or T replacement (group 5 or 6). Interestingly, normal females given T (group 3) show a significant reduction in sprouting while normal females given E (group 2) are not significantly different from controls. Males show no significant decline in sprouting when castrated (group 4) as compared to controls and no significant change in either the normal or castrated state when administered T . However, normal male animals given E (group 2) show a significant decline in reactive growth when compared to all other male groups. We previously found that E replacement increased sprouting in the female. We now find that T also aids the overectamized female. Normal animals of both sexes show a decrease in the sprouting response, when administered the opposite sex hormone.

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297.18 **CA1 MOSSY FIBERS IN THE RAT: DE NOVO PROJECTION?** **T.M. Cook and K.A. Crutcher.** Dept. Anatomy, Univ. of Utah Sch. Med., Salt Lake City, UT 84132.

In the adult rat hippocampal formation, dentate granule cell axons, or mossy fibers (MF) innervate CA3 but not CA1 pyramidal cells. Mossy fibers are only found in rat CA1 following removal of the CA3 pyramidal cells during a restricted period of development (PN 3-6). To determine the requirements for eliciting this developmental rearrangement we analyzed the pattern and amount of CA3 cell loss resulting in CA1 MF. We also sought to determine whether MF are transiently present in CA1 during development as might be predicted from the parcellation hypothesis (Ebbesson, Cell Tissue Res. 218, 1980).

Neonatal rats were injected at various ages with kainic acid (KA). Some also received a commissurotomy with or without KA. Serial (16 um) sections were processed for Nissl and Timms histochemistry. CA1 and CA3 cells were counted and the area measured on Nissl-stained sections through the dorsal hippocampal formation. HRP injections were made in pups ranging from PN 1-4 with 24 hr. survivals. Rats at the same ages were perfused according to the Timm method to visualize MF development.

Mossy fibers were present in CA1 only in those cases in which CA3 cell loss was 80% or greater. The pattern of CA3 cell loss was also important since CA1 MF only occurred when there was a gap in the CA3 cell layer. Commissurotomy did not affect the incidence or extent of CA1 MF although this procedure reduced pup mortality following KA injection. Complete removal of CA1 and CA3 cells resulted in MF within the hilar region only. HRP injections into the dentate gyrus did not result in any granule cell or MF labeling. Nor was there any evidence of CA1 MF during this period of development based on the Timm-stained material.

These results indicate that the presence of CA1 MF is dependent on the amount and pattern of target cell loss during a specific period of development. If, in fact, CA1 MF are not transiently present during development, this rearrangement would represent the establishment of a new projection in the rat CNS. This may reflect a phylogenetically primitive connection since CA1 MF have been reported in the hedgehog (Gaarskjaer et.al, Brain Res. 237, 1982). (Supported by grant BNS 81-03678 from NSF.)

297.19 APPARENT SPROUTING OF STRIATAL DOPAMINERGIC TERMINALS AFTER 6-HYDROXYDOPAMINE LESIONS OF ADULT RAT. S.P. Onn, M.J. Zigmond, E.M. Stricker and T.W. Berger. Depts. of Biological Sciences & Psychology, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

Intraventricular injection of 6-hydroxydopamine (6-HDA) in adult rats results in a medial-lateral gradient of dopaminergic (DA) terminal loss throughout the striatum when examined with histofluorescence techniques one week postlesion. The greatest loss occurs in the periventricular, medial striatum where few, if any, residual DA terminals are observed. Biochemical assay of regional striatal punches from the same brains confirmed an average DA depletion to 2% of control levels in the medial striatum and a smaller DA depletion in lateral striatum. When animals from the same lesioned group were examined 15 weeks postlesion, however, a greater density of fluorescent terminals was evident in the medial striatum, and corresponding DA content was 8% of age-matched control levels. Greater DA concentrations also were observed in the central and lateral striatum. These differences may represent lesion-induced sprouting of DA nerve terminals from the substantia nigra (SN).

Considering the well-known topographical nature of nigrostriatal projections, we attempted to determine whether the terminals in medial striatum visualized 15 weeks postlesion originate from medial SN cells normally innervating that region or from SN cells normally innervating more lateral striatal areas. HRP (20 nl; 10%) was injected into the medial striatum of rats given intraventricular 6-HDA 1 or 15 weeks previously. The distribution and number of HRP retrogradely-labeled neurons within the ventral tegmental (VTA)-SN area was determined. Material from animals injected one week postlesion showed only 2% of the number of retrogradely-labeled neurons found in vehicle-injected control animals (375±13, n=6). In contrast, material from animals injected 15 weeks postlesion revealed 10% of the number of cells labeled in age-matched controls (469±25, n=6). This difference in retrogradely-labeled neurons is consistent with the possibility of terminal regrowth. Nearly all of the labeled neurons at 15 weeks postlesion were still confined to VTA and medial SN, suggesting that the normal topographical nature of nigrostriatal afferents was present after the lesion. These findings may represent a regeneration of previously lesioned axons from SN, or collateralization of SN axons which survived the initial lesion.

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297.20 HYPERINNERVATION OF STRIATUM BY DORSAL RAPHE AFFERENTS AFTER DOPAMINE-DEPLETING BRAIN LESIONS IN NEONATAL RATS. S. Kaul*, T.W. Berger, E.M. Stricker and M.J. Zigmond. Psychobiology Program, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Dopamine-depleting brain lesions in 3-day-old rats produced by intraventricular 6-hydroxydopamine (6-HDA) leads to an increase in striatal serotonin (5-HT) content when measured one month later (Stachowiak et al., Brain Res., 291, 1984). The lesion-induced increase in 5-HT levels occurs primarily in the rostral striatum, where 5-HT terminals normally are distributed sparsely. Cell bodies in the brainstem raphe nuclei are known to provide 5-HT innervation of the intact rat striatum. To determine whether 6-HDA lesion-induced increases in 5-HT represent a sprouting of raphe afferents to striatum, we compared the quantity and distribution of retrogradely-labeled neurons in the raphe nuclei after HRP injection in the striatum. Three-day old rats were injected intraventricularly with either 6-HDA (N=5) or its vehicle (N=4). When animals reached 5-6 weeks of age, HRP (0.05 µl, 20%) was injected into the rostral striatum. Tissue was processed using standard procedures. Every other section (40µ) through the substantia nigra was examined for HRP-containing processes, as was every section through the raphe nuclei.

For animals injected with vehicle alone, a mean total of 261 (range = 222-309 per animal) weakly labeled HRP-positive neurons was observed in the dorsal-most portion of the dorsal raphe n. No other raphe nuclei were seen to contain retrogradely filled cells. Neurons within the pars compacta of the substantia nigra were heavily labeled with HRP, and dense anterogradely transported product was visible in the pars reticulata. In marked contrast, material from animals injected with 6-HDA contained 4-5 times as many HRP-filled neurons in the dorsal raphe n. (mean = 1,111, range = 958-1331 per animal). In addition, labeled neurons in lesioned animals were distributed throughout both the dorsal and ventral portions of the dorsal raphe, though not in other raphe nuclei. The 6-HDA injections specifically and completely lesioned the dopaminergic substantia nigra; there was a virtual absence of retrogradely labeled neurons in the pars compacta, and yet dense anterogradely labeled terminals were present in the pars reticulata. These data demonstrate a lesion-induced proliferation of raphe projections to striatum that may underlie the increased levels of striatal 5-HT occurring after dopamine-depleting lesions of neonatal rat brain. Supported by NS19608.

297.21 NOREPINEPHRINE IN THE IPN: EFFECTS OF LOCUS COERULEUS LESION. W. Battisti*, B. Levin* and M. Murray. Dept. of Anatomy, The Med. Coll. of PA and Neurology Service, VA, East Orange, NJ.

The IPN is an unpaired midline nucleus which receives bilateral input from several sources. Its major afferents, the paired fasiculi retroflexus (FR), show considerable plasticity. Lesions to one FR elicit sprouting by remaining FR axons in subnuclei in the IPN where the two FRs converge. The IPN also receives converging input from the norepinephrine (NE) containing axons arising from the paired loci coeruleus (LC). Plasticity of LC neurons is well documented. We wished to map the normal LC projection into the IPN, determine the extent of overlap of the two LC projections, and then determine whether a lesion of one LC would induce compensatory sprouting by the undamaged LC in adult rats.

LC lesions were made by injections of 6-OHDA into one or both LC and animals killed 2 weeks or 2 months later. Some brains were reacted for fluorescence using the glyoxylic acid method. In others, NE content of IPN was determined using HPLC. Histochemical analysis indicates that the LC projection to the IPN is concentrated in the central subnucleus. Little fluorescence is seen in those subnuclei (lateral, intermediate) where sprouting by FR axons has been described. Bilateral LC lesions produce a marked and permanent loss of NE. Histochemical observations show loss and little recovery or redistribution of fluorescence after chronic bilateral LC lesions. Unilateral LC lesions produce a permanent depletion of NE of about 50% in the IPN. The amount of fluorescence appears to be partially and equally diminished throughout the central subnucleus, suggesting that the projections of the two LC overlap.

The projection of paired LC to the unpaired midline IPN is thus symmetrical, bilateral, and overlapping. Destruction of 1 LC however, does not seem to be the appropriate stimulus for sprouting by the undamaged LC neurons. The properties of the LC projection to IPN thus differ from those of the FR projection which is similarly symmetrical and in part overlapping and biochemically homogeneous, and where destruction of 1 of the paired inputs elicits sprouting by the remaining system.

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REGENERATION II

298.1 IMPLANTATION OF FETAL CORTEX AND SPINAL CORD INTO ADULT SPINAL CORD: INITIAL RESPONSES. Jerald J. Bernstein and Dennis W. Hoovler. Lab. CNS Inj. and Regen., V.A. Med. Ctr., Washington, D.C. and Depts. Neurosurg. and Physiol., George Wash. Univ. Sch. Med., Washington, D.C. 20422.

The implantation of fetal CNS into the brain of adult hosts has been sucessful, formed new connections, had trophic effects on the host and has resulted in amelioration of behaviors lost by prior lesion. In contrast, fetal CNS transplantation into the spinal cord has only been studied very recently. In the following experiments E11 fetal cortex or spinal cord was implanted between the dorsal horn and dorsal column of the spinal cord of 36 adult male Sprague-Dawley rat hosts. Hosts were perfused for light and electron microscopy 1,3,7, and 10 days later. The donors were E11 of time pregnant Sprague-Dawley females. Controls were cesarean delivered pups from donor females (2 per group) matched for days after implantation and two controls per group for surgical implant controls. Insertion of the implanting needle in controls resulted in the formation of a cyst in the dorsal columns. Implantation of the fetal tissue by pressure injection from a 30 gauge needle in the spinal cord results in the formation of a cyst. The phagocytic activity in the cyst is most active at 1 day and removes most of the debris from the presumably fluid filled cyst. Between 1 and 3 days the transplanted E11 cortex and spinal cord form multiple circular neuroepithelia. This is an indication that the transplant was in pieces when implanted. At 7 and 10 days these neuroepithelia have sedimented ventrad in the cyst and contain many dividing spongioblasts and neuroblasts. Neurons with differentiated nuclei and occasionaly with well differentiated cytoplasmic organelles were observed. At later postimplantation, times the transplant grows to fill the cyst and has overlapping neuropil at the isthmus of the spinal cord due to earlier sedimentation. Supported by the Veterans Administration.

298.2 IMPLANTATION OF FETAL RAT CORTEX INTO REGENERATING PERIPHERAL NERVE OF ADULT RAT. D. W. Hoovler and J. J. Bernstein. Lab. of CNS Injury and Regeneration, V. A. Medical Center, Washington, D.C. and Depts. of Neurosurgery and Physiology, George Washington University School of Medicine, Washington, D.C., 20037.

Implantation of undifferentiated fetal CNS tissue into the nervous systems of adult hosts is an effective method of analyzing the possible replacement of lost function due to injury or disease of the adult nervous system. Transplants of fetal cortex and spinal cord survive up to four months (length of experiment) in regenerating peripheral nerve (Bernstein, J. Neurosci. Res., 1983) and from one to two months in degenerating peripheral nerve (Bernstein and Tang, Brain Res., 1985). In the present study, morphological aspects of the implantation of 11 day old fetal (E11) rat cortex into regenerating peripheral nerves of adult rats were analyzed during the first three weeks following implantation. Under Chloropent anesthesia, 45 adult male Sprague-Dawley rats (200-300g) had the epineurium of the nerve to the biceps femoris muscle crushed and incised, the perineurium minced and E11 cortex injected, just distal to the crush site, via a glass micropipette attached to a 50 µl syringe. Rats were sacrificed 1, 3, 5, 7, 10, 12, 14, 17 and 21 days postimplantation (DPI) and processed for light or electron microscopy. Implants were compared to cortices from age matched pups as a control. At early sacrifice times (1-7 DPI), immature neurons and neuroglia were found dispersed or in neuroepithelial-like structures throughout the degenerating nerve. At 7-14 DPI, neurons and neuroglia appeared to be more mature and were interspersed among groups of regenerating host peripheral nerve axons. At the latest sacrifice dates (14-21 DPI), aggregates of mature neurons and neuroglia, surrounded by neuropil containing a rich synaptic bed, were observed among regenerated host peripheral nerve axons. Degenerating neurons were observed at all postoperative dates. Thus, fetal cortical implants survived, grew and differentiated throughout the postoperative period. This study provides a model for analyzing trophic and electrophysiological aspects of fetal nervous tissue implantation into adult peripheral nerves. (Supported by the Office of Naval Research, CB-030-820505, and the Veteran's Administration).

298.3 **THE IMPLANTATION OF RAT FETAL SPINAL CORD INTO INJURED AND UNINJURED ADULT HOST SPINAL CORD AND PYRAMIDAL TRACT.** U. Patel and M.R. Wells*. Adult Psychiatry Branch, NIMH, Saint Elizabeths Hospital and *The Veterans Administration Hospital, Washington, D.C. 20032

It has been demonstrated that E11-15 day rat embryo implants of fetal spinal cord can survive and to some extent differentiate when inserted into adult host spinal cord (Patel and Bernstein, J. Neurosci. Res., 9:303-310, 1983). We have investigated several conditions of the host spinal cord for their effect on implant survival and differentiation. Conditions included overlaying the implant on the host pia, and insertion of the implant into various lesions including a dorsal mid-line myelotomy, spinal hemisection, a lesion of the dorsal columns, and a medullary pyramidal tract lesion. Implants consisted of embryo (E11-15) spinal cords labelled with ^{3}H-thymidine which had been dissected out free of meninges and connective tissue. At time intervals of 2, 3 and 4 weeks, 2, 3, 6, 8 and 12 months after implantation the hosts were perfused and examined histologically.

Implants survived under all conditions of implantation, although the extent of differentiation and the cells surviving varied. Younger embryos (E11-12) seemed to survive best. Fetal cells, even from spinal overlays were observed invading the host spinal cord. The migration of cells into the host occurred along the neuroglial septa of the spinal cord toward grey matter. After reaching the grey matter, the cells seemed to disperse individually. There was evidence for the migration of cells rostro-caudally upto several centimeters depending on the duration of the implant in the host. In some instances, structures resembling central canals were observed in the implanted tissue. In one instance morphogenic differentiation of a fetal spinal cord occurred next to the host vertebral column.

Cavitation or enlargement of the host spinal central canal was occasionally observed near the implant site but scar formation around the implant was reduced. At longer time intervals (6-12 months), some of the implants appeared to degenerate. The incorporation of the implant was occassionally accompained by degeneration of host axons. However, axons of undefined origin were usually present in the implant under all conditions.

These data demonstrate that spinal cord implants can survive in injured and uninjured host spinal cord upto one year. Careful study will be necessary before such techniques may be considered for the repair of injured host spinal cord.

298.4 FETAL RAT SPINAL CORD TISSUE TRANSPLANTED INTO RAT SPINAL CORD: IMMUNOCYTOCHEMICAL CHARACTERIZATION OF THE HOST-GRAFT INTERFACE. J.R. WUJEK AND P.J. REIER. Dept. of Anatomy, Univ. of Maryland Sch. of Med. Baltimore, MD 21201.

Our laboratory has been studying the transplantation of fetal spinal cord into injured adult spinal cord. In this study, we have used immunocytochemistry to examine the glial composition of the transition zone between host and implant. Donor tissue from E14 rat fetuses was transplanted into a cavity created by hemisection of the adult rat spinal cord. The host was sacrificed 1-8 months later and frozen sections were obtained from the spinal cord region containing the implant. The sections were then stained with antiserum to Glial Fibrillary Acidic Protein (GFAP). Although each implant generally filled the lesion cavity, none were observed whose surfaces were completely apposed to the host spinal cord. Thus, areas of contact between each implant and the spinal cord varied from moderate to extensive. Such regions exhibited an intimate fusion between the host and donor tissue without an obvious intervening cellular interface. Where the implant was directly apposed to host spinal grey matter, the transition zone exhibited only a moderate GFAP-immunoreactivity. A slightly more intense GFAP-immunoreactivity was seen in the transition zone adjacent to white matter, which probably reflects gliosis associated with degeneration of fiber tracts. On the other hand, when the implants were either separated from the host by cysts or contacted by mesodermal tissue (e.g. from the overlying meninges), GFAP-immunoreactivity was more intense. In these instances, a dense layer of astrocytic cytoplasmic processes lined the periphery of the implants. Such glial encapsulation was also observed along the injured surfaces of spinal cords which did not contain an implant. These observations were confirmed quantitatively, using a video-based image analyzer to measure the area of tissue occupied by GFAP immunoreactive elements. Thus, in this study, only modest gliosis was observed when host and donor tissues were closely apposed in the injured spinal cord. In contrast, intense astrocytic reactivity and development of a distinct glial limiting membrane occurred when the surfaces of either the transplant or host spinal cord were exposed to non-CNS environments. (Supported by NS 13836 to P.J.R.)

298.5 TRANSPLANTATION OF EMBRYONIC AND NEONATAL DORSAL ROOT GANGLIA INTO THE SPINAL CORDS OF ADULT AND NEONATAL RATS. S. Williams, B.T. Himes*, K. Winkler* and A. Tessler. Depts. of Neurology and Anatomy, VA Medical Center and The Medical College of Pennsylvania, Philadelphia, PA 19129.

Although dorsal root ganglion (DRG) neurons have been reported to survive transplantation into the brain of recipient animals (Ranson, 1914), it is not yet clear whether they survive transplantation into the spinal cord or, if so, whether they develop or maintain characteristics typical of DRG cells. In the present investigation we have examined the capacity of DRG neurons taken from fetal and newborn animals to survive in the spinal cord of host animals and examined the transplants for the presence of substance P (SP). Donor DRG neurons labeled with ^{3}H-thymidine were dissected from fetal (E14) or neonatal (day 0) Sprague-Dawley rats, dissociated with trypsin, and transplanted into subtotal lesions made in the spinal cords of newborn (0-3 days) or young adult (250gm) rats. After survivals of 4-12 weeks, recipients were sacrificed by the intracardiac perfusion of fixative, and frozen sections were cut transversely at 16u on a cryostat. Adjacent sections were stained with cresyl violet or labeled on slides with the peroxidase-antiperoxidase technique for the presence of SP-immunoreactivity.

Fetal and newborn DRG neurons survived in the spinal cords of both neonatal and adult animals whether transplanted into cervical, thoracic, or lumbar regions. Sections stained with cresyl violet contained large and small DRG neurons with well defined nuclei and nucleoli clustered in groups together with smaller, non-neuronal cells that stained more darkly. In some cases the transplanted DRG cells were closely apposed to or found within the white matter of the host spinal cord. Sections of fetal transplants stained with SP-antiserum showed immunoreactive perikarya among the small DRG neurons as well as immunoreactive processes. These results suggest that fetal and newborn DRG neurons can survive transplantation into the spinal cord of newborn and adult recipients and that specific characteristics of some cells develop or are maintained through the survival period.

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298.6 IN-VITRO REGENERATION OF LEECH PERIPHERAL AXONS. L.C. Soileau and W.J. Thompson. Dept. of Zoology, University of Texas, Austin, TX 78712.

The regenerative abilities of the peripheral axons of the medicinal leech (Hirudo medicinalis) were examined in organ culture. Ganglia with attached peripheral nerve roots were explanted together with a piece of tissue taken from leech body wall. The severed end of one or more of the nerve roots was inserted into this piece of "target" tissue. After various periods of culture (5-40 days) the reinnervation of the "target" by identified sensory and motor neurons was examined by electrophysiological methods. Responses in the peripheral mechanosensory axons were recorded in response to mechanical or electrical stimuli applied to the tissue. Each mechanosensory neuron retained its stimulus modality. The receptive fields formed were discrete but small, .5x.5mm - 3x3mm. The touch (T) cells had the largest receptive field, followed next in size by the pressure (P) cells. The nociceptive cells (N) were never seen to form a large receptive field. Only the region near the tip of the severed nerve evoked responses in the N cell. Extent of sprouting was determined by intracellular injections of Lucifer Yellow. The receptive field size determined by electrophysiological methods correlated exceptionally well with the dye injections results.

Neither positional specificity nor target tissue specificity was maintained under culture conditions. Whereas the touch and pressure mechanosensory neurons normally innervate discrete portions of skin, it was seen that the T and P cells would sprout into skin regardless of any positional origin. In additon to sprouting in skin, T and P responses were evoked even if the peripheral axons were implanted into a target consisting of only muscle tissue or of the connective tissue which normally surrounds the leech CNS.

Even though severed motor axons reinnervate muscle tissue specifically in vivo (Van Essen and Jansen, J. Comp. Neurol. 171;433, 1977). we have never seen any muscle reinnervation in culture. Dye injections show that the motor axons do sprout extensively in the peripheral roots. The reasons for the lack of success of reinnervation of muscle tissue by motor neurons remain unknown.

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298.7 THE RESPONSE OF DORSAL ROOT AFFERENT FIBERS TO DORSAL FUNICULUS LESIONS IN DEVELOPING RANA CATESBEIANA TADPOLES. H.L. Campbell, M.S. Beattie, and J.C. Bresnahan. Dept. of Anat. and Div. of Neurosurg., and Neurosci. Res. Lab., Ohio State Univ. Coll. Med., Columbus, OH 43210.

The response to axotomy of dorsal root axons traveling in the dorsal funiculus was tested at several developmental stages in bullfrog tadpoles. Unilateral lesions of the dorsal columns were placed just rostral to the lumbar enlargement at early (stages I-VI of Taylor and Kollros, 1946), intermediate (stages XII-XIV), or late (stage XVII-XX) periods during hindlimb development. After varying periods of time for recovery (4 to 14 weeks), axons of the eighth dorsal root ipsilateral to the lesion were filled with HRP and after 12-18 hours, tissue was processed using the CoCl-enhanced DAB reaction. Surgical procedures were accomplished while animals were anesthetized by hypothermia and/or MS 222.

Injury-filling of dorsal roots with HRP allowed visualization of the patterns and morphology of groups of axons as they approached the lesion site, as well as their distribution and morphology caudal to the lesion. Axons growing into (or remaining within) the lesion site appeared disorganized. Some, presumably regenerating fibers could be seen following the surface of the cord under the pia. We have not observed significant numbers of fibers penetrating beyond the lesion site, but our technique labels fibers for only 10-15 mm total. A common feature of these experiments was alterations in axonal morphology in the spinal gray caudal to lesions. Large caliber axons could be followed from the dorsal columns entering the spinal gray along tortuous paths unlike the trajectories noted in control experiments. Such fibers, which often exhibited very large **en passant** and terminal swellings, were similar to those recently described by Liuzzi and Lasek after regeneration of dorsal roots in adult frogs. Some bulbous elements exhibited fine diameter extensions reminiscent of filipodia.

In order to examine the effect of blastema implants on regeneration, autologous grafts were taken from tail amputation sites and placed into the dorsal funiculus. Preliminary results show that labelled dorsal root axons penetrate the graft. EM studies of lesioned and implanted dorsal column preparations are currently underway. (Supported by NS-10165)

298.8 RECONNECTION OF THE EIGHTH NERVE FIBERS AFTER TRANSECTION IN THE RED-EARED TURTLE. D. Marbey and R.H. Browner. Dept. of Anatomy, New York Medical College, Valhalla, NY 10595

The eighth nerve was transected in the red-eared turtle. Bones over the inner ear and 8th nerve were exposed. The nerve was carefully transected between the ganglion and the brainstem without disruption to the vascular supply. The area was roofed over with dental cement. The operated turtles were housed in a constant environment (25°C) for the appropriate survival time. Animals were allowed to survive for 22 days, 42 days, 52 days, 64 days and 77 days. Evaluating the effects of the transections entailed reanesthetizing the animal to surgically visualize either the cochlear duct (CD) or the acoustic tubercle (AT). HRP was injected into either structure. An additional 3 days per age was allowed prior to sacrificing. Following perfusion the brains were removed, embedded in gelatin albumin, and frozen-sectioned in the coronal plane at 40 µm intervals. Every third section was stained for HRP with the TMB Method (Mesulam, 1982). Alternate sections of the 25 and 67 day survivors were placed in 10% formalin for 1 week and then stained for degenerating terminals and axons (Fink and Heimer method #2).

The 25 day survivor group did not display any HRP reaction in the primary auditory nuclei or the 8th nerve following a CD injection. Degeneration byproduct was evident in the 8th nerve and the nucleus magnocellularis (NM) and the nucleus angularis (NA). Some degeneration byproduct was evident in the nucleus vestibularis lateralis (NL)(Marbey and Browner, 1984, In Press).

The 50, 55 and the 80 days survivors which received acoustic tubercle injections demonstrated HRP filling in the 8th nerve ganglion and in the 8th nerve fibers projecting to the acoustic tubercle. The 67 day survivors which received (CD) injections demonstrated HRP reaction product around the somata and within the cells of the NM and NA. Some HRP filling was evident in the NL. Sequential sections of the 67 day survivors stained for degeneration demonstrated only a small amount of terminal degeneration.

Results indicated that turtles surviving 50, 55, 67 and 80 days had presynaptic and terminal axonal filling of HRP. Those surviving 25 days following surgery did not. Restoration of the 8th nerve connections to the primary auditory nuclei was indicated by the presence of HRP reaction product in these structures. Supported by the Whitehall Foundation #48-259.

298.9 THE SEPARATION OF ADULT AXONS IN NERVE ROOTS AND PERIPHERAL NERVES ENZYMATICALLY AND THE RESULTANT AXONAL BEADING. T. Spagnolia, W. Levy, R. Rumpf. Division of Neurosurgery, University of Missouri School of Medicine, Columbia, Missouri 65212.

Methods of studying axons in adult tissue have usually involved teasing a few viable axons out of an adult nerve preparation, where a small percentage of the preparation was successfully separated without damage. In order to study axonal injury processes we are using a technique in which we enzymatically dissect adult axons, with a high percentage of undamaged axons achieved. In the method a rat is anesthetized with sodium pentothal and an incision made from the abdomen to the proximal hind limb medially. With dissection, a superficial branch of the sciatic nerve is exposed and the neurovascular pedicle of this nerve is dissected out along with a muscle supplied by the neurovascular pedicle, keeping its function intact. The rat is then placed in a special sling on the stage of an inverted phase contrast microscope and the pedicle placed from the rat across an isolation and perfusion chamber. The attached muscle of the pedicle is in a second more distal chamber where it is kept in a saline solution. A portion of the neurovascular pedicle which is in the working chamber has had the nerve separated from the artery and vein through the course of the chamber. The nerve is placed in a slot in a coverslip preparation and can be examined with the inverted microscope. The temperature is controlled to 37°. A multi-roller peristaltic pump is used to perfuse past the nerve in the chamber. The substrate medium is a modified Brimijoin's solution. Initially a calcium-free solution is perfused for thirty minutes, then a .5% collaganse solution for approximately one hour, and a .1% trypsin solution for approximately 1 hour. It can be observed that gradually the epineureum of the nerve disappears allowing visualization of the axons with the phase contrast microscope. Axons dissect out from the nerve and between 30% and 80% of the nerve is disassociated into axons.

The axons are usually without external signs of damage and axonal contours and myelination indistinguishable from axons visualized in the preparation before dissection. When damage occurs it is usually in the form of axonal beading. The beading appears to arise at three locations: in the paranodal area, between, and at the Schmidt-Lanterman clefts. Under continuous observation, the beading can often be seen to start at a Schmidt-Lanterman cleft and enlarge. The development of this method was done for use in a study of axonal injury processes where the axons dissected in the adult living nerve prep are manipulated into close alignment with each other after sectioning to study the influence of this on growth cone guidance.

298.10 REGENERATION IN THE CEREBELLUM OF THE POSTNATAL AND ADULT MOUSE IN LONG TERM CULTURES. H.F. Johnston*, H.M. Sobkowicz, G.L. Scott*, and C.V. Levenick*. Depts. of Neurology, Psychiatry, and Anatomy. University of Wisconsin, Madison, Wisconsin, 53706.

Twenty cerebellar explants from the adult and 15 from the 9- to 12-day old mice were cultured up to 12 days in vitro. Neuronal and glial outgrowth appeared within the first few days. Ultrastructurally, the first signs of regeneration--glial growth and repair--are similar in cultures of all ages examined. The most striking event is the formation of a continuous, multilayered, intricate glial cover at the periphery of the explant. First, the glial cover separates the explant from the substrate; next, the glial processes invade juxtaposed neural tissue; finally, some cells climb toward and cover the upper surface, separating the tissue from the feeding solution. The survival, maintenance, and regrowth of neuronal elements depend critically on the presence of the glial cover; in the areas where the glial enclosure is not complete, degeneration prevails. At least three types of astroglial cells; phagocytizing glia and macrophages participate in the formation of the glial cover. Specialized membrane junctions may be seen between juxtaposed glial processes. Among synaptic contacts, the easiest to recognize are axodendritic synapses on the thorns of Purkinje dendrites; most of them are made by the varicosities of parallel fibers. Some abandoned thorns retain their postsynaptic density. Many neuronal profiles form boutons filled with synaptic vesicles or growth cones. The latter show sometimes a mixture of growth vesicles and synaptic vesicles. There are few axosomatic synapses. The cells having nuclei and cytoplasm characteristic of neurons survive without apparent preference for cell type. Purkinje cells, Lugaro cells, large Golgi cells, and granule cells have been all tentatively identified. Among glial cells, large Golgi epithelial cells, astrocytes with clear cytoplasm or those displaying filamentous content are abundant. Astroglia in mitosis was observed. Phagocytizing glial cells and macrophages are present throughout the explant. In summary: 1) Adult cerebellar tissue can survive explantation and show signs of regeneration and regrowth. 2) The regeneration of the nervous tissue depends critically on the development of a glial cover that corresponds closely to the glia limitans of the intact animal. 3) The neural and glial relationships during regrowth and regeneration are similar in young and adult tissues. Supported by NSH Grant NS15061.

298.11 EARLY AXONAL AND DENDRITE SPROUTING IN AXOTOMIZED LAMPREY NEURONS. S. A. Mackler*, H.-S. Yin, M. E. Selzer, (SPON: D. H. Silberberg). Department of Neurology, University of Pennsylvania, Philadelphia, PA 19104

Axotomized giant interneurons (GIs) of larval sea lampreys show two types of neurite outgrowth: 1) neurites regenerate from the proximal axon stump, 2) long axon-like neurites sprout from anomalous locations on the soma or dendrites. The second type must change direction if they are to project normally. We have previously observed that in aminals surviving more than 4 weeks post-axotomy 70-85% of the distal ends of both types of neurites are oriented rostral and contralateral to the cell body, which is the normal axon projection for GIs. Is this directional specificity due to retraction after initial random neurite outgrowth? To answer this we examined neurite orientation during the first 4 weeks using intracellular injection of HRP.

In the first fourteen days, while axons were dying back, no evidence of sprouting was observed and the distal ends of all axons remained in their proper projection path. Between two and four weeks 64% of all regenerating neurites were correctly oriented.

During the first four weeks 77% of neurites with anomalous origins were normally oriented at their distal ends, compared to 85% after four weeks.

At two weeks, GIs showed a transient 30% increase (p 0.05) in the average number of primary and secondary dendrites per cell. However, at no stage did they show a profusion of randomly oriented long neurites. Therefore, directional specificity of regeneration is not achieved by selective retraction from among many randomly oriented axon-like neurites. Instead a directionally selective trophic influence seems to guide the growth of individual fibers.

298.12 EFFECTS OF AXOTOMY ON LAMPREY INTERNEURONS. H.-S. Yin, S. A. Mackler*, M. E. Selzer. Department of Neurology, University of Pennsylvania, Philadelphia, PA 19104

Axotomy is known to produce retrograde morphological changes which may reflect signals for regeneration. In large larval lampreys axotomy produced the following changes in the rostrally projecting giant interneurons (GIs): 1) up to a three-fold increase in diameter of the cell body, 2) eccentricity of the nucleus, 3) changes in dendritic morphology (discussed in a second abstract), 4) loss of cytoplasmic chromophilia and 5) a perinuclear chromophilic shell. Using the cell diameter as a quantitative index, the retrograde reaction showed centrifugal movement of about 0.5 mm per day away from the point of axotomy with a latency of 6 days. Lateral cells, which are caudally projecting interneurons showed similar morphological changes.

Intracellular recordings from GIs at two weeks post-transection showed a 2½ fold decrease in the frequency of spontaneous synaptic potentials compared to untransected controls (0.70/sec vs. 1.71/sec). Thereafter, the frequency increased, so that after four weeks it was 2½ fold greater (4.59/sec) than in controls. The amplitudes of spontaneous EPSPs showed a reversible increase, reaching a maximum of 3-fold at 4 weeks (1.53 mV ± .33 SE vs. 0.52 mV ± 0.08 for controls). Resting membrane potentials (RMP) were unchanged during the first 4 weeks, but thereafter decreased from 60.9 mV ± 1.9 SE to 41.1 mV ± 1.6.

The changes in morphology and RMP in GIs were similar to those previously described for another cell type, the primary sensory dorsal cells, except that cell diameters decreased in the latter. In both cases, the maximum morphological changes occurred at approximately the time of earliest axonal regeneration across the transection site.

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298.13 ANATOMICAL AND FUNCTIONAL STUDIES OF AXONAL REGENERATION FROM NEURONS IN THE SOMATOSENSORY CORTEX OF THE ADULT RAT BRAIN. M. Vidal-Sanz*, M. Rasminsky and A.J. Aguayo. Neurosciences Unit, Montreal General Hospital and Department of Neurology and Neurosurgery, McGill University, Montreal, Canada.

Axons from neurons in several different regions of the adult rat central nervous system have been shown to elongate extensively through peripheral nerve grafts inserted into the brain (Nature 296:150,1982). Brainstem neurons regenerating axons into peripheral nerve grafts remain functionally active and can be excited and inhibited by afferent stimulation (Soc. Neurosci. Abstr. 9:698,1983). Here we examine axonal regrowth from nerve cells in the somatosensory cortex of these animals.

In adult Sprague-Dawley rats weighing approximately 250 gm, a 3.5 cm long segment of autologous sciatic nerve was removed and inserted into the ipsilateral cerebral cortex (0-2 mm caudal and 4-6 mm lateral to bregma), in close proximity to the region of the cortical "barrel" fields that normally receive inputs from mystacial and non mystacial vibrissae in the face and mouth. The other end of these nerve grafts was sutured and left blind-ended between the scalp and subcutaneous tissues.

From 2 to 6 months after grafting, the extracranial portion of the graft was exposed and transected close to its distal tip. Horseradish peroxidase was applied to the cut end to label retrogradely the soma of the neurons whose axons had grown into the graft. The location of these neurons was determined in whole mounts of these cortices and also in cross sections of the brain. Labelled neurons were found in different layers about the "barrels". In all animals these cells were in close proximity to the intracerebral tip of the graft.

We recorded unitary activity from single regenerated axons teased from the grafts, in some cases observing centrifugal spontaneous activity. Stimulation of the muzzle contralateral to the graft gave rise to both excitatory and inhibitory responses.

These results indicate that as is the case in the brainstem, some somatosensory cortex neurons that regenerate axons into peripheral nerve grafts remain responsive to afferent stimulation.

298.14 AGE OF NEURAL IMPLANT AND INDUCTION OF LIMB REGENERATION. B. F. Sisken, I. Fowler* and E. Barr*. Wenner Gren Research Laboratory and Dept. Anatomy, Univ. of Kentucky, Lexington, KY 40506.

We are studying the effects of neural tissue implants on amputated 4 day chick embryo limbs. In this study, we describe the response obtained when the age of the implant was varied, and the morphological appearance of the youngest donor tissue after induction of new limb segments had occurred. Right wing buds were amputated at the future elbow joint. Experimental embryos had a segment of 2 day neural tube or 3,4,6 or 8 day spinal cord implanted longitudinally into the limb stump. Control embryos were implanted with 7 day heart tissue or were not implanted. Previous studies (Fowler and Sisken, 1982) demonstrated that 29.3% of the embryos implanted with 2 day neural tube regenerated middle and distal segments (Type III response), while 27.7% showed no response (Type I), and 43% an intermediate response (Type II). Our present studies indicate that donor implants obtained from 3, 4 or 6 day embryos produced similar but slightly lower percentages (22-26%) of Type III regenerates; no regeneration occurred with 8 day neural implants, or in any control group. We have concluded that younger donor neural tissue has a greater chance of survival, resulting in greater induction of new limb growth. To determine the degree of development of the implant in situ, we have started investigating the tissue at the EM level. Two-day neural tube implanted into the limb was examined 9 days after insertion in Type III embryos, and compared with that in Type I embryos. Differentiation of closely-packed neuronal cells and processes in the implant were significantly developed in Type III limbs, while the neurons in implants of Type I limbs were immature and contained many processes separated by large spaces. These results indicate that differentiation of the implanted tissue also plays a role in determining the success of limb induction. (NIH NS 19788-02).

298.15 REGENERATION OF AXONS IN PARENT AND TRIBUTARY NERVES. Chung-Bii Jenq and Richard E. Coggeshall. Marine Biomedical Inst. and Dept. of Anat. and Physiol. and Biophys., Univ. of Tex. Med. Branch, Galveston, TX 77550.

Previously we showed that when nerves regenerate, axonal numbers in tributary nerves are dependent on the type of lesion that interrupts the parent nerve. Here we show 1) that axonal numbers in the distal stump of the parent are also dependent on the type of transecting lesion and 2) that the ratio of axons that regenerate in the distal stump as compared to the numbers in the tributaries that arise from that stump is the same in some situations and different in others.

The rat sciatic nerve is the parent and the nerve to the medial gastrocnemius muscle (NMG) and sural nerve (SN) are the tributaries. Sciatic nerves are 1) crushed (C), 2) transected (0), 3) transected and 4mm of nerve removed (4) and 4) transected and 8mm of nerve removed (8). The mean axon counts from nerves on the unoperated (Un) and operated (Op) sides 8 weeks after the lesion are shown below.

	Sciatic Distal Stump				S.N.				N.M.G.			
	MY		UN		MY		UN		MY		UN	
	Op	Un	Op	Un	Op	Un	Op	Un	Op	Un	Op	Un
C	10052	7998	12730	16539	1312	947	2696	3183	394	298	778	458
0	13276	8107	9752	15322	1667	1062	2035	3557	513	335	752	475
4	13586	8396	10447	14899	1396	1008	1917	3385	450	312	530	425
8	7046	7905	7638	15573	491	973	906	3456	236	317	463	403

Thus the numbers of axons that regenerate are different in the distal stump of the sciatic nerve when the transecting lesion is different. Second, the myelinated axons in the NMG and the myelinated and unmyelinated axons in the SN regenerate in concert with the axons in the distal stump of the sciatic for the crush, 0mm and 4mm transections. By contrast proportionately fewer axons regenerate in the tributary nerves in the 8mm transection. This seems to imply that there is a certain gap length beyond which the organism does not regulate axon numbers in regenerating tributary nerves. By contrast, there are proportionately more unmyelinated axons in the NMG than in the distal stump in all paradigms. We do not yet know the meaning of this finding but it implies that separate axonal populations regenerate differently in our paradigms. Supported by NIH grants NS10161, NS17039, NS07377 and NS11255.

298.16 REGENERATION OF SCIATIC NERVE WITH AND WITHOUT AN AUTOGRAFT. L. Wilson*, Chung-Bii Jenq and R.E. Coggeshall. (SPON: H.J. Nauta). Marine Biomed. Inst. and Dept. of Anat. and Physiol. and Biophys., Univ. of Tex. Med. Branch, Galveston, TX 77550.

Here, we report the effects of a sciatic nerve autograft upon the numbers of axons that regenerate through the gap when an 8mm length of sciatic nerve is removed.

Under nembutal anesthesia, an 8mm length of sciatic nerve segment was removed from an adult rat. The stumps were then sliced in a silicon tube and each secured by a single stitch. In the grafted group, the removed sciatic segment was placed between the stumps. Eight weeks later, the axons were counted in the middle of the tube and in the distal stump. The table below shows means and standard deviations of these counts.

	In The Tube		Dist. Stump	
	No-Gr.	Graft	No-Gr.	Graft
Myel.	4642 ±1226	8285 ±1732	7046 ±2262	9238 ±2287
Unmy.	10446 ±1853	10783 ±2355	7638 ±3271	13163 ±4509

Note that the number of myelinated axons in the tube in the grafted group is greater than that in the non-grafted group ($p < 0.02$). Also note that when no graft is done, myelinated axon numbers in the distal stump are greater than in the tube ($p < 0.02$, paired t-test). For the unmyelinated axons, there is no statistically significant difference in the tube. In the distal stump, however, there are relatively fewer unmyelinated axons if there is no graft and relatively more if the graft is done. We thus conclude that the sciatic autograft in the tube results in more myelinated axons crossing the gap, and reduces the branching of regenerating axons as they enter the distal stump. Behavioral tests will be necessary to see if these changes are beneficial.

Supported by NIH grants NS10161, NS17039, NS07377 and NS11255.

298.17 A LONGITUDINAL ANALYSIS OF THE EFFECTS OF IONIZING RADIATION ON THE HIPPOCAMPAL FORMATION OF THE RAT. Robert B. Wallace, Philip Jasin*, John Gustafson*. Lab. of Developmental Psychobiology, University of Hartford, West Hartford, CT 06117.

A number of earlier investigations had indicated that focal neonatal X-irradiation of the rat hippocampus was capable of reducing the numbers of postnatally proliferating granule cells of the dentate gyrus (Altman and Das, J. Comp. Neurol., 124: 319, 1965; Wallace, Kaplan and Werboff, Exp Brain Res., 24:1, 1976). In all of these studies, however, relatively short survival times were employed. A study reported in the literature in 1970 dealing with focal X-irradiation of the cerebellum and involving survival times of up to two years indicated that behavioral abnormalities apparent in pre-weaning observations tended to disappear in middle age and in parallel with this change was a partial replacement of the number of granule cells in the IGL (Wallace, Daniels, and Altman, Develop. Psychobiol., 5 (1): 33, 1970). In an effort to determine if a similar potential might exist in the hippocampus, the following experiment was carried out. 62 Long-Evans hooded rats (Rattus nurvegicus) from our animal colony were used. A 250 kV Kelekett deep therapy unit was used as the source of X-irradiation. A target-to-animal treatment distance of 39 cm was employed with a dose rate of 50 rad/min. and a field size of 13.5 x 7 cm. A 2 mm copper sheet was used for filtration. Pups were wrapped in plastic tubes and placed in a lead-shielded lucite block with a slit that allowed only the hippocampal portion of the head to be irradiated. Irradiation treatments began on postnatal day 2. On the 2nd and 3rd days after birth, animals received 200 rad. Thereafter, irradiation received was 150 rad on alternating days (5th through 15th day postnatally). Nonirradiated control animals were used to provide base line data. 3 experimental and 3 control animals were sacrificed at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 months. The number of granule cells in the dentate gyrus in matched sections was counted at 400x magnification. Results indicated that in the control animals, numbers of granule cells remained fairly constant over all ages sampled. In the experimental animals, there was an initial degranulation of about 50% against the control baseline. In the 1 to 2 year old groups, some recovery was noted in terms of numbers of cells but not to control levels. A decrease was observed in the 2 year + groups. Some transient reconstitutive capacity of the proliferative matrix is suggested by these data and a parallel is noted to the results earlier observed in the degranulated cerebellum.

298.18 CULTURED PNS CELLS SUPPORT PERIPHERAL NERVE REGENERATION THROUGH TUBES IN THE ABSENCE OF DISTAL NERVE STUMP. H.D. Shine, P.G. Harcourt* and R.L. Sidman, Departments of Neuropathology, Harvard Medical School and Neuroscience, Children's Hospital, Boston, MA. 02115.

Mouse axons will grow through a $\leq$ 10mm gap formed when a peripheral nerve is severed and the cut ends placed in opposite ends of a tubular prosthesis. The presence of the distal nerve stump within about 10mm of the proximal stump is essential for axon regrowth (Williams, et al., Brain Res. 293:201 '84). We examined the effect of cultured dorsal root ganglion cells (DRG; fibroblasts, Schwann cells, and sensory neurons) in permitting or stimulating peripheral axonal regeneration through tubes. Dissociated DRG cells from mouse embryos were grown in a collagen matrix (Vitrogen, Flow Labs) and placed in a polyethylene tube 7mm long, 1mm i.d. Sciatic nerves of adult mice were cut, the proximal stump was inserted into one end of the tube, and either the distal stump or a glass plug was inserted into the other end so that a 5mm chamber separated proximal stump from distal stump or plug. Tubes in control mice were saline-filled or contained collagen matrix without cells. After 4 wks the implants were fixed and cross-sectioned for light microscopy at points 1.5mm from each end. Only myelinated axons have been scored to date, but the specimens will allow EM examination to delineate the growth of unmyelinated axons. When the distal stumps were attached, myelinated axons were present 1.5mm from the distal ends in control and experimental animals; this suggests that either the cultured cells had no influence on regeneration or the distal stump masked the cells' effect. However, when the distal end of the tube contained a glass plug, axons regenerated and became myelinated to the distal end only when cultured cells had been added. Control tubes containing collagen matrix without cells supported formation of some myelinated axons in the proximal end only, and axons did not regrow at all into saline-filled tubes. DRG neurons were not observed in any of the implants; they appear to have died and did not contribute the axons observed within the tubes. These results indicate that cultured PNS cells will mimic at least some of the effects of the distal stump on axonal regeneration and offer a means for identifying their cellular source and nature. Supported by Dysautonomia and King Foundations and NIH grants HD18655 and NS20821.

298.19 EPENDYMA-MESENCHYME INTERACTION DURING SPINAL CORD REGENERATION. S.B. Simpson, Jr. and K. Pollack*. Dept. of Biochemistry, Molecular and Cell Biology, and the Neuroscience Program, Northwestern Univ., Evanston, IL 60201.

During tail regeneration in the lizard Anolis, ependyma cells regenerate as a continuous epithelial tube and guide hundreds of regenerating central nerve fibers. If, however, the lizard cord is injured at mid-back levels, no regenerative response occurs. Histologically, the end result is identical to that exhibited by the injured mammalian cord.

We report here results suggesting that the failure of cord regeneration at mid-back levels is due to the absence of an appropriate mesenchymal (connective tissue) wound environment. Ablation of the normally regenerating cord in the tail, followed by tight suturing of the incision (preventing overt blastema formation), results in the failure of cord regeneration. Segments of normally regenerating tail cord, autografted to an ablation zone in the mid-back region, likewise fail to regenerate in their sparse connective tissue wound environment. Segments of normally nonregenerating cord from mid-back regions autografted to the tail, under conditions that allow blastema formation, exhibit a regenerative response comparable to that of normal tail cord. These results are consistent with the ependyma-mesenchyme hypothesis of spinal cord regeneration previously articulated (Simpson, S.B., Jr. 1983 IN: Spinal Cord Reconstruction. C. Kao, R. Bunge and P. Reier, eds., pp. 151-162, Raven Press).

Attempts to create a connective tissue wound environment that will support cord regeneration at mid-back levels are currently underway. (Supported by NIH grant NS20970)

298.20 MATRIX MODIFICATION ENHANCES NERVE REGENERATION WITHIN A SILICONE CHAMBER. L.R. Williams and S. Varon. Dept. of Biology, Sch. of Med., Univ. Calif. San Diego, La Jolla, CA 92093.

We have previously defined the spatial-temporal progress of rat sciatic nerve regeneration across a 10 mm gap within an 11 µl silicone chamber (J. Comp. Neurol. 218:460) and have delineated stages of the sequence crucial to regeneration success: 1) fluid accumulation; 2) formation of a continuous, coaxial fibrin matrix; 3) replacement of the matrix by an appropriate cellular environment; and 4) axonal elongation and myelination. We now find that fluid accumulation and matrix formation can be modified by increasing chamber volume and prefilling the chamber with saline at the time of implantation. Such modifications enhanced regeneration and resulted in the formation of a 3-fold larger diameter nerve that contained 3-fold more endoneurial area and 3-fold more axons. Chambers with a 25 µl and 75 µl volume were implanted empty (E) or prefilled with saline (PF) and the spatial-temporal progress of their regeneration was compared to that in control E11 and PF11 chambers. The E25 and E75 chambers took longer to fill with fluid than E11 chambers and fluid accumulation was greater from the proximal stump. By 1 wk, a coaxial continuous fibrin matrix had formed that had a prominent proximal-distal taper. The taper was more pronounced in E25 and E75 chambers due to significantly larger matrix diameters in the proximal chambers. These observations indicated that the proximal stump was a preferential source of chamber fluid and matrix precursors. At 3 wks, Schwann cell migration and axonal regeneration were retarded in the larger chambers compared to E11 chambers. The retardation correlated with the presence of a small diameter, avascular organization of squamous circumferential cells. Prefilling chambers with saline permitted immediate diffusion of nerve stump exudate throughout and resulted in the formation of a larger diameter non-tapering 1 wk matrix containing smaller caliber and more dispersed fibrin fibers. At 3 wks, regeneration in the PF11 chamber was not different from E11 chambers but was enhanced in the PF25 system: 3-fold more axons had regenerated across the chamber. Replacement of matrix by migrating cells was retarded in PF75 chambers. Thus modification of the early intrachamber events has large impact on subsequent cell and axonal behavior.
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298.21 Vascular Patterns in Intact Rat Brain and in Brains Which Contain Implants of Fetal Tissue: Studies Using Micropangiography and Ink Injection. L.A. Paul , R. Gibbs, L. Braun , W.H. Oldendorf, C. Cotman. Dept. of Psychobiology, UC Irvine, Irvine, CA 92717 and Brentwood VA Hosp., Los Angeles, CA 90073

Knowledge of patterns of microcirculation in the brain is relevant for understanding neovascularization following CNS injury as well as neuron-vascular interactions during development and their functional relationships. To this end, we studied vascular patterns in intact rat brains and in those of animals at several time points following implantation of fetal CNS tissue from donors aged E16-18 days. Using stereotaxic methods, implants of approximately 4mm^3 were introduced into the brains of male anesthetized rats which had previously received a knife cut transecting entorhinal cortex. A second group was composed of rats which received the knife cut only. At various times (1 day, 1 week, 1 month, 2 months) following implantation, animals were perfused with fixative and their brains injected with either a radiopaque material (Micropaque) or India ink with gelatin dissolved in it. Unoperated rats of similar size comprised a third group.

Material injected with Micropaque was cut in 1000 micron sections and exposed to a beam of 40 KeV for 200 seconds in a table-top X-ray unit (Faxitron). The India ink brains were cut at 50 microns on a freezing microtome and counterstained. The tissue was then subjected to qualitative and quantitative analysis. The X-ray material, on thick sections, provided information about the pattern of larger (>10 microns) vessels, while the India ink allowed us to visualize capillaries as well.

Preliminary impressions of longer-term implanted brains indicate that, in many cases, the capillary/neuron ratio is poorer than in intact cortical regions. It is possible that this ratio relates simply to neuronal density. In addition, the vessels within and immediately approaching the implant appear thicker, of more uniform diameter, and less tortuous, than in adjacent or homologous cortical regions. The pial penetrating vessels, so notable in neocortex, are disrupted near the implant. Gross microcirculatory patterns, as revealed in X-Ray photographs, appear remarkably undisturbed.

Several questions remain to be answered: 1) does the capillary pattern in the implant conform more to that of the host, or to that of the embryonic donor?; 2) from what major trunks do the vessels reaching the implant arise; 3) what are the respective roles of donor and host in angiogenesis in this model of CNS repair following injury?

298.22 PRODUCTION OF A SPECIFIC ANTISERUM THAT BINDS TO DEGENERATING FIBER TRACTS IN THE RAT CNS. C. Brian McGuire*, G. Jackson Snipes*, Jeanette J. Norden, and John A. Freeman. Dept. of Anatomy, Vanderbilt Medical School, Nashville, TN 37232.

Injury to nerve fibers in the central nervous system stimulates a number of reactive events by glia and other cellular elements. At the molecular level, one of the major cellular responses is the secretion of a soluble 37kd protein following nerve injury (Skene & Shooter, PNAS, 1983). We have purified this protein by preparative 2D gel electrophoresis, and have raised a highly specific rabbit antiserum to it, as verified by Western blot analysis. The 37kd antigen was then immunocytochemically localized in frozen sections of rat brains, following different CNS lesions.

Anesthetized adult rats received unilateral lesions of either the optic nerve or primary motor cortex. After one to three weeks, the rats were reanesthetized, the brains removed and cut into transverse 10um sections in a cryostat. The sections were fixed in 2:1 chloroform/methanol and placed in hydrogen peroxidase/methanol for 30 min (4°C) to block endogenous peroxidase activity. Following rehydration and paraformaldehyde fixation, the 37kd antigen was localized using a standard PAP technique, at a 1:500 primary antiserum dilution.

In rats with optic nerve lesions, the antiserum bound specifically to the contralateral optic tract, and to a much lesser degree to the ipsilateral optic tract. Binding was also found in retinal fiber layers in the contralateral LGN and superior colliculus. In rats receiving lesions of motor cortex, binding was found in the ipsilateral corticospinal tract through the entire brainstem. No preferential binding to degenerating fiber tracts was observed in control experiments in which brain sections were incubated with non-immune serum and antiserum pretreated with the 37kd protein. Thus binding occurs specifically in both sensory and motor CNS pathways undergoing early degenerative changes following axonal injury. Although the function of the 37kd protein has yet to be determined, these results suggest that induction and secretion of this protein by neuroglial cells associated with damaged axons is an early and general response to injury, and might play a role in the subsequent degenerative or regenerative metabolic response of neurons.

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298.23 TO WHAT EXTENT IS NORMAL AUTONOMIC FUNCTION RESTORED FOLLOWING REGENERATION OF LESIONED PREGANGLIONIC SYMPATHETIC FIBERS? STUDIES ON THE PINEAL GLAND. J.R. Lingappa and R.E. Zigmond. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115

It is known that peripheral sympathetic nerves regenerate following a lesion, but it is not known if normal end-organ function returns. We have examined this question by looking at the recovery of function of the rat pineal gland after bilaterally lesioning the cervical sympathetic trunks (CST). The pineal gland is innervated by neurons in the superior cervical ganglia (SCG) that are in turn innervated by preganglionic fibers of the CST. Following bilateral crushing of the CST or sham operations, the activity of the pineal enzyme serotonin N-acetyltransferase (NAT) was measured. The activity of this enzyme normally exhibits a circadian rhythm, which is under sympathetic neural control, with peak activity occurring at night. Thirty-six hours after both CST were crushed, peak night NAT activity was decreased to 1% of the value in sham-operated controls. One hundred days after the lesion, enzyme activity had recovered to only 13% of control values. A time course experiment indicated that the recovery of NAT activity had reached a plateau by this time. Choline acetyltransferase (ChAT) activity within the SCG was negligible three days after the lesion and recovered to 50% of the sham-operated value by 100 days. Sectioning the regenerated CST abolished pineal NAT and ganglionic ChAT activities, indicating that the recovery of these enzyme activities depends on regenerated CST fibers. We compared the ability of normal and regenerated CST fibers to increase pineal NAT activity when electrically stimulated. When both CST were stimulated at 10 Hz for 3 hrs, enzyme activity was increased to the same extent in both groups, reaching near peak night levels. Thus, although regenerated CST fibers fail to raise pineal NAT activity during the rat's nighttime, they are in fact capable of raising NAT activity if electrically stimulated. These data raise the possibility that the regenerated fibers are adequate in number and efficacy to elevate pineal NAT activity. A reason for their failure to restore pineal function could be that following regeneration many of the postganglionic fibers that innervate the pineal gland are being driven with a pattern of electrical activity that is not appropriate for producing a normal rhythm in NAT activity. Further study is needed to determine if this is the case. Supported by USPHS grants NS 17512 and MH 00162.

298.24 FUNCTIONAL RESTORATION OF CIRCADIAN RHYTHMS FOLLOWING SUPRACHIASMATIC NUCLEUS TRANSPLANTS IN THE RAT* Raúl Aguilar*, René Drucker Colín, Federico Fernández Cancino*, Fernando García,* and Federico Bermudez Rattoni. Centro de Investigaciones en Fisiología Celular, UNAM, México.

In recent years the procedure of grafting fetal brain tissue into adult rats has provided means of correcting the behavioral deficits produced by either brain lesions or congenital abnormalities. Moreover morphologically several authors have shown that the grafted tissue becomes differentiated and integrated into the host brain. The purpose of this study is to demonstrate that the loss of the circadian rhythm of drinking due to suprachiasmatic lesions can be restored by grafting the homologous fetal area into the adult rat.

Wistar male rats were placed in cages specially designed for measuring drinking behavior. Every time a rat touched the water dispenser, this generated a pulse. This pulse was picked on line by a PDP 11-34 computer. Each rat's drinking behavior was thus recorded for 48 hours. Following the recording of the normal rhythm, the suprachiasmatic nucleus of all rats was electrolytically lesioned. Upon recovery, their drinking rhythm was again recorded for 5 weeks. On the following day 10 rats were grafted with the suprachiasmatic nucleus of 17 day old fetal rats. The effect of the graft was followed for 2 months. At the end of the experiments histological analysis of all rats was carried out. The results of these experiments showed that loss of the circadian periodicity of drinking by suprachiasmatic lesions, was restored by grafting fetal suprachiasmatic tissue. The effect is illustrated below.

Histological analysis will be presented showing the reinnervation of this area. The results of these studies show that transplanted neurons can correct functional and morphological CNS deficiencies. *Supported by a Grant from the "Fundación Ricardo Zevada".

298.25 ALLEVIATION OF ESTROGEN-INDUCED HYPERPROLACTINEMIA BY HYPOTHALAMIC TISSUE TRANSPLANTS CONTAINING DOPAMINERGIC NEURONS. G.W. Arendash and P.C.K Leung. Lab. of Neuroendocrinology and Exp. Neurology, Dept. of Biology, Univ. of South Florida, Tampa, FL 33620 and Dept. of Ob/Gyn., Univ. of British Columbia School of Medicine, Vancouver, BC, Canada V6H 3V5.

Prolactin-secreting pituitary tumors can be induced in young rats through prolonged estrogen administration. Recent evidence (Science 218:684, 1982) suggests that such tumors are associated with a degeneration of tuberoinfundibular dopaminergic (TI-DA) neurons, which normally inhibit prolactin secretion by the ant. pituitary's lactotrophs. In this study, chronic hyperprolactinemia was induced in young, ovariectomized Fischer 344 rats through silastic capsule implants of 17-B estradiol, placed subcutaneously for one month prior to removal. Rats with such estrogen-induced hyperprolactinemia then received transplants of neonatal hypothalamic tissue (containing TI-DA neurons) or amygdala (control) tissue, placed either bilaterally within the hypothalamus, or within the third ventricle. Blood samples were obtained one month after transplantation via external jugular tap and prolactin concentrations measured by radioimmunoassay. Of the four animals receiving third ventricular implants of hypothalamic tissue, two showed dramatic reductions in blood prolactin levels, to 4% and 23% of mean values for control animals. Also, four of the seven animals receiving bilateral implants of hypothalamic tissue showed 33-59% reductions in their prolactin levels compared to mean values for control animals. Thus, approximately half of all hyperprolactinemic females receiving hypothalamic tissue transplants experienced an alleviation or elimination of estrogen-induced hyperprolactinemia.

Follow-up catecholamine (CA) histochemistry indicated a bright fluorescence in the median eminence of animals bearing effective hypothalamic transplants; this, in comparison to a weak CA fluorescence within the median eminence of animals remaining hyperprolactinemic with ineffective grafts.

These data suggest that TI-DA neurons, within hypothalamic grafts effective in reducing blood prolactin levels, innervated the host median eminence and became functional. Thus, an alleviation/elimination of estrogen-induced hyperprolactinemia appears possible through a reconstitution of normal dopaminergic inhibition of prolactin secretion by means of transplanting TI-DA neurons.

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299.1 CHARACTERIZATION OF THE 37KD PROTEIN ASSOCIATED WITH NERVE DEVELOPMENT AND INJURY. G. Jackson Snipes* and John A. Freeman. (SPON: A.M. Burt) Dept. of Anatomy, Vanderbilt University School of Medicine, Nashville, TN 37232.

Soluble extracellular proteins comprise a significant portion of the environment surrounding neurons and glia, and may play an important role in neural development and regeneration. Rat glial cells selectively release an acidic 37kd protein during development (Snipes, et al, PNAS, 1984) and following nerve injury (Skene & Shooter, PNAS, 1983). To characterize this protein we have produced a highly specific rabbit antiserum against the 37kd protein isolated from injured adult rat sciatic nerves. Wesetrn blot analysis, immunoprecipitation and two-dimensional gel electrophoresis (2D-PAGE) analysis have indentified four distinct forms of the 37kd protein which can be independently isolated. Injured adult rat optic nerves, injured adult sciatic nerves and neonatal sciatic nerves all secrete distinct, immunologically related 37kd proteins. A fourth form is found as a normal component of rat serum.

The relationship between these different forms was clarified by biochemical analysis of the 37kd protein in the sciatic nerve. Soluble extracellular proteins isolated from excised rat sciatic nerves are distributed into intravascular and extravascular compartments. Plasma proteins are representiative of those found in the intravascular compartment. To examine the extravascular compartment, rats that had previously received a unilateral sciatic nerve crush were perfused with Dulbecco's phosphate buffered saline (D-PBS) to eliminate intravascular proteins. Then, both the normal and injured sciatic nerves were removed, cut into 5mm segments, and shaken with D-PBS to wash out the soluble extracellular proteins, which were then compared to those found in normal plasma using 2D-PAGE. The plasma 37kd protein is excluded from the extravascular space around normal sciatic nerves, whereas the 37kd protein obtained from injured nerves accumulates in the extracellular, extravascular space surrounding the nerves. We conclude that there are multiple forms of the 37kd protein, and that the neurally derived form is related to an as yet unidentified form normally present in rat serum. The different forms of the 37kd protein are compartmentalized in such a way as to allow both a systemic and a local action of the protein, which may be associated with nerve growth and development. Supported by NIH grants EY01117 and NS18103 to JAF.

299.2 Peptide homology between the intermediate filament proteins of neuronal and nonneuronal origin from goldfish optic nerve. N. Schechter and W. Quitschke. Department of Psychiatry and Behavioral Sciences, SUNY at Stony Brook, New York 11794.

Vigorous regeneration of the goldfish optic nerve is observed after crush. An analysis of optic nerve proteins indicates that the predominant proteins in the goldfish optic nerve have a molecular weight of 58K. These proteins can be separated by two-dimensional gel electrophoresis and are observed as a series of isoelectric variants. They have been designated as ON proteins (Quitschke and Schechter, J. Neurochem., 41 (1137-1142) 1983). Previous studies have shown that ON_1 and ON_2 are neurofilament proteins whereas ON_3 and ON_4 are intermediate filament (IF) proteins of nonneuronal origin (Quitschke and Schechter, J. of Neurochem., 42 (569-576) 1984). Since different cells within the same nerve tissue express an apparently homologous series of IF proteins, a structural analysis of these proteins with respect to their peptide fragments was performed.

Both unlabeled and ^{35}S-methionine labeled proteins were used in these studies. The proteins were separated in the first dimension by isoelectric focusing and hydrolysed directly within the gels. Reagents employed for proteolysis, in separate experiments were V8 (S. aureus protease), chymotrypsin, and cyanogen bromide. The resulting peptide fragments were separated in the second dimension (SDS) and visualized by either Coomassie blue staining, silver staining or autoradiography.

Preliminary results indicate that there are common peptide fragments between all of the ON proteins. In addition, fragments are observed which seem to be unique to the 58K neurofilament proteins and to the 58K intermediate filament proteins. These results suggest that the similarities between these proteins may be related to a common structural function. The differences may be related to cellular specialization and function which reflects the regenerative capacity of the goldfish optic nerve. (This research was supported by grant EY05212 from the NIH).

299.3 Intermediate filament proteins: differential expression between the optic nerve and spinal cord in mammals and lower vertebrates. W. Quitschke and N. Schechter, Dept. of Psychiatry and Behavioral Science, SUNY at Stony Brook, New York 11794

The intermediate filament (IF) protein composition of the goldfish visual system is dissimilar to analogous tissues in mammals. The predominant intermediate filament proteins of the goldfish optic nerve have molecular weights of 58K. These proteins can be separated into a series of isoelectric variants which have been designated as ON_1 - ON_4. (Quitschke and Schechter, Journal of Neurochem. 42 (1984) 569-576). ON_1 and ON_2 are neurofilament proteins whereas ON_3 and ON_4 are nonneuronal intermediate filament proteins. The concentration of ON_1 and ON_2 varies with the degeneration and regeneration of the goldfish optic nerve whereas ON_3 and ON_4 are largely unchanged. In addition, subcomponents of ON_1 and ON_2 are specific to the retinotectal pathway. Because of this specificity, a study was undertaken to determine the expression of intermediate filament proteins in optic nerve and spinal cord of various species (rat, hamster, goldfish, frog and newt) which were selected with regard to their developmental characteristics and regenerative capacity. Intermediate filament proteins in optic nerve and spinal cord were analyzed by two-dimensional gel electrophoresis and identified by reacting them with anti-IF antibodies. In vitro incubations of excised optic nerve in the presence of ^{35}S-methionine distinguished between neuronal and nonneuronal intermediate filament proteins. The proteins of the intermediate filament complex in the two tissues for rat and hamster were similar. The typical neurofilament triplet and GFAP were observed. Vimentin was more concentrated in the optic nerve than in the spinal cord. The goldfish, newt and frog contained neurofilament proteins in the 145 - 150K range and in the 70 - 85K range. In addition, prominent neurofilament proteins in the 58 - 62K molecular weight range were found in all three species. In contrast to mammalian species, the goldfish, newt and frog displayed extensive heterogeneity between optic nerve and spinal cord in the expression of both neuronal and nonneuronal intermediate filament proteins. The distinctive presence of low molecular weight neurofilament proteins and their high concentration in the optic nerve and spinal cord of these nonmammalian vertebrates suggests that the expression of this class of IF proteins may be related to the regenerative capacity observed for these species. (This research was supported by grant EY05212 from the NIH).

299.4 PURIFICATION AND CHARACTERIZATION OF A DENERVATION-INDUCED NERVE SHEATH RELEASED PROTEIN M.J.Ignatius, H.W.Muller, J.H.P.Skene and E.M.Shooter. Dept. of Neurobiology, Stanford Univ., Stanford, Calif. 94305.

The PNS grafting experiments of Aguayo have suggested a supporting role of Schwann cells in stimulating growth of neurons both in the CNS and PNS. We have been examining whether glia might exert this influence through the secretion of soluble, extracellular proteins that "condition" or prime the denervated sheath for growth. To date it has been shown the two to four weeks after either cutting or crushing the sciatic nerve in rats several soluble secreted proteins are induced in non-neuronal sheath cells. Most prominent among these is a 37K dalton protein, first described by Skene and Shooter (PNAS 80:4169;1983) and Politis et al (Brain Res. 273:392;1983). We have isolated and begun to characterize this protein in order to study its possible role in the growth and maturation of neurons.

37 K was purified from adult rat sciatic nerves that had been crushed 3 weeks prior to their removal. The portion of the nerve distal to the site of injury was removed, partially minced and the secreted, soluble extracellular proteins were obtained by incubation of the tissue in nutrient medium. The medium containing the 37 Kd protein was dialysed and loaded onto a DEAE 52 ion exchange column in 50mM sodium phosphate buffer pH 6.0, washed with several column volumes of 100mM phosphate, and the 37K enriched fractions eluted early during a linear gradient from 150mM to 500mM phosphate. The pooled 37K fractions were concentrated, resuspended in 0.1% SDS, 2mM DTT, and 10% glycerol and the proteins separated by preparative polyacrylamide gel electrophoresis. This final step yielded a homogeneous sample of denatured 37K as revealed both in coomassie and silver stained 2-dimensional polyacrylamide gels.

Rabbit antibodies were raised to the denatured 37K protein and the specificity of the serum for denatured 37K was confirmed by antibody probing of protein blots. Antibodies to 37K also recognize a protein of molecular weight 110Kd not found in control nerves, that is abdunant in crushed nerves. Using this antibody to purified rat 37k we are asking whether the induction of this protein is a common feature of both regenerating and developing systems from a variety of sources. (Supported by NIH grants (NS 04270 and NS 20178) and the Isabella Niemala Fund)

299.5 DEVELOPMENTAL PROGRAM OF PROTEIN EXPRESSION IN RAT RETINAL GANGLION CELLS. S. Bock, G. J. Snipes, J. J. Norden, J. A. Freeman, (SPON: L. H. Aulsebrook), Dept. of Anatomy, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232. Growth and development of the optic nerve is intimately associated with a programmed sequence of induction of rapidly transported proteins in the retinal ganglion cells (RGC's; Bock et al., Neurosci. Abst., 9:1099, 1983). In order to study this developmental sequence of protein expression in greater detail, we have developed a computerized method to analyse proteins resolved by 2D PAGE and visualized by fluorography in late embryonic and early neonatal rats, which corrects for local labeling. At these early ages (4 days before birth to 8 days after birth) the usual method of labeling rapidly transported proteins by injecting ^{35}S-methionine intravitreally results in substantial non-specific labeling due to the increased permeability of immature membranes to the amino acid, with subsequent vascular uptake and diffusion. This results in labeling of proteins synthesized by optic nerve glial cells in addition to proteins synthesized by RGC's and transported down the nerve. To solve this problem we obtained matched pairs of gels at a succession of developmental time points, where each pair consisted of: a) rapidly transported proteins and locally labeled glial proteins following intravitreal injection of radioisotope, and b) glial proteins only. The latter was obtained by separating the RGC axons from their cell bodies and labelling the surviving glial cells ^{35}S-methionine *in vitro*. After densitometric scanning (512x512 resolution), the proteins on each set of gels were resolved and quantitated with a new segmentation algorithm, and using a comparison program, glial contaminants were deleted from the RGC gels. The remaining RGC proteins can be divided into 3 classes on the basis of their rates of synthesis during development. This rate remains constant for the largest class of proteins. The second class, which includes a 20kd and a 43kd protein, is expressed during neonatal development, while the third class, which includes 3 prominent 29kd proteins, appears only with maturation.

We conclude that the use of computer analysis provides an effective method to unambiguously identify developmentally regulated, rapidly transported RGC proteins in a complex mixture of RGC and glial proteins. Many of these RGC proteins appear to be associated with specific developmental stages, and appear to merit further investigation. Supported by NIH Grants EY01117 and NS18103.

299.6 A "GROWTH-ASSOCIATED PROTEIN" (GAP-43) IN DEVELOPING AND SEVERED AXONS OF THE HAMSTER PYRAMIDAL TRACT. Pate Skene and Katherine Kalil. Dept. of Neurobiology, Stanford University, Stanford, CA 94305; and Dept. of Anatomy, University of Wisconsin, Madison, WIS 53706.

Skene and Willard (J. Cell Biol. 89: 86-106, 1981) have proposed that certain "growth-associated proteins" (GAPs) are necessary for some stages in axon growth, and that failure to induce these proteins limits regeneration in the CNS of higher vertebrates. The hamster pyramidal tract offers a strong test of this hypothesis, because pyramidal tract axons will regenerate if they are severed in hamsters less than two weeks old, but not if they are severed in animals more than three weeks old (Kalil and Reh, JCN 211: 265-275, 1982). The "GAP hypothesis" predicts that in hamsters less than two weeks old, pyramidal tract neurons either synthesize GAPs constitutively or are induced to synthesize GAPs in response to axotomy. The hypothesis predicts further that in animals older than three weeks, GAP synthesis is largely repressed and is not re-induced in response to axotomy. Our current results confirm these predictions for one "growth-associated protein", GAP-43.

We injected 35-S-methionine into the sensorimotor cortex of normal hamsters at various ages (4-32 days old) and allowed 2-3 hours for rapidly transported proteins to travel into pyramidal tract axons. A small region of medulla encompassing the pyramidal tract was then removed and quick-frozen, and the labeled proteins were later separated by two-dimensional electrophoresis and detected by fluorography. In neonates, we found a 43kd membrane protein which co-migrates in electrophoresis with GAP-43 from regenerating toad optic nerves. Labeling of this hamster "GAP-43" is maximal in animals 4-8 days old, declines slightly during the second postnatal week, and then declines sharply (greater than 10 fold) between postnatal days 12 and 21. In preliminary experiments, pyramidal tracts were severed in 24 day old hamsters, and axonally transported proteins were labeled 7-8 days later by injecting 35-S-methionine into sensorimotor cortex as before. We found no evidence for increased synthesis of GAP-43 in response to axotomy in these animals, supporting the proposition that abortive regeneration in the adult mammalian CNS involves failure of axotomized neurons to re-induce GAPs. We are now investigating whether GAP-43 synthesis is increased or prolonged by severing pyramidal tract axons in 4-12 days old hamsters, when regeneration does occur.

299.7 CHANGES IN TRANSPORTED RETINAL PROTEINS CORRESPOND WITH THE TIME OF INTERACTION BETWEEN REGENERATING OPTIC FIBERS AND THE TECTUM IN GOLDFISH John E. Marsh and Myong G. Yoon Department of Psychology, Dalhousie University, Halifax, N. S. Canada B3H 4J1.

A group of rapidly transported proteins (M. W. 110-140 Kd) in regenerating optic fibers of goldfish increase in the presence of the tectum compared with those regenerating without the tectum. In the present experiment we varied the period between axotomy and reinnervation to determine whether timing of the optic fiber-tectum interaction would correspond to the changes in this specific group of retinal proteins. The left optic nerve (projecting to the right tectum) and the left optic tract (projecting to the left tectum) were simultaneously crushed in all fish. This manipulation results in a disparity in the time required for tectal reinnervation by the tract-crush side relative to the nerve-crush side by approximately 9 days. The complement of rapidly transported proteins in the two groups of regenerating optic fibers were compared by differentially labeling them with 3-H and 14-C proline at times ranging from 8-37 days after axotomy and separated by one-dimensional gel electrophoresis.

At early post-operative periods (12-15 days) there was an increase in 110-140 Kd proteins on the tract-crush side relative to the nerve-crush side. This trend was reversed at later time points (18-37 days); relative increases were found on the nerve-crush side. These results demonstrate a correspondance between the time of optic fiber-tectum interactions and changes in the rapidly transported 110-140 Kd retinal proteins. Another group of proteins (M. W. 42-46 Kd) showed a relative increase in the nerve-crush side during early periods (8-15 days). This change in 42-46 Kd proteins disappeared at later periods (18-37 days). This result suggests that the regeneration of the axons of retinal ganglion cells after optic nerve crush require a greater amount of the 42-46 Kd proteins than that after a simultaneous optic tract crush during the early periods.

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299.8 PHOSPHOPROTEINS OF THE GOLDFISH TECTUM DURING OPTIC NERVE REGENERATION. L.I. Benowitz and K.L. Moya. Dept. Psychiatry (Neuroscience), Harvard Med. Sch.; McLean Hosp., Belmont, MA 02178.

Regeneration of the optic nerve in lower vertebrates is associated with a shift in the pattern of proteins that are synthesized in the retinal ganglion cells and transported intra-axonally to the nerve terminals. Among the most prominent of these changes is a 100-fold increase in a group of acidic proteins, Mr=44-49 kilodaltons (K), that are conveyed down regenerating optic fibers in the rapid phase of axonal transport (Benowitz and Lewis, J. Neurosci. 3:2153, 1983; Skene and Willard, J. Cell Biol. 89:86, 1981). In the present study we examined whether these proteins are related to B-50, an acidic 48K phosphoprotein of mammalian presynaptic membranes (Zwiers et al, J. Neurochem. 34:1689, 1980), while also examining the changes in intrinsic phosphoproteins of the optic tectum associated with denervation and reinnervation. Membrane and cytosol fractions of the tectum were phosphorylated at various times following optic nerve surgery. Calcium-calmodulin and c-AMP-dependent phosphorylation followed the protocol of Zwiers et al, except for the substitution of [^{35}S]-PO_3S^*-γ-ATP as a source of labeled phosphate.

In the absence of Ca^{++}, the incorporation of label into the tectal membrane fraction was restricted primarily to a prominent component at 49K. In the presence of Ca^{++} + calmodulin, several other membrane proteins, most having molecular weights in the 50-60,000 dalton range, became heavily phosphorylated. This pattern did not change over the course of optic nerve reinnervation. None of the tectal phosphoproteins corresponded on 2-D gels with rat B-50 which was run in parallel, nor with the 44-49K proteins of the regenerating optic nerve terminals, from which we conclude that the latter proteins are not B-50. Among the soluble proteins of the optic tectum, increased phosphorylation was found for soluble components between 45-50K at day 10 post-surgery, which then declined once the optic nerve began to return. The pattern of phosphorylation of the soluble proteins did not depend on the presence of Ca^{++}-calmodulin or c-AMP. The time-dependent changes in soluble phosphoproteins may play a role in regulating the metabolism of the deafferented optic tectum, or perhaps even in inducing changes in remote neurons. (Support: NINCDS RO1-16943 and the American Paralysis Association.)

299.9 [^{3}H]ACTINOMYCIN D BINDING TO THE NUCLEI OF AXOTOMIZED NEURONS: A COMPARISON OF THE CNS AND PNS NUCLEAR REACTIONS TO AXON INJURY. M. R. Wells and M. F. Hall.* Neurochemistry Research Laboratory, Veterans Administration Medical Center and Department of Physiology, George Washington University, Washington, D.C. 20422

We recently introduced an autoradiographic method to study [^{3}H]actinomycin D (Act. D) binding to nuclei in tissue sections as an indicator for changes in chromatin structure and RNA synthesis (Wells, M.R.; Anat. Rec. 208: 193A, 1984). In the axotomized spinal ganglia of rats, nuclear binding of [^{3}H]Act. D occurred as a biphasic response over time. Binding to nuclei significantly above normal at 1-3 days and 7-11 days postoperation was observed. Below normal levels were detected at 5-7 days and 14 days. The timing of the response was related to the distance of the lesion from the cell body. For a CNS injury model, layer V pyramidal cells of a rat somatomotor cortex were examined after medullary pyramidal tract lesions. Comparisons were made using the contralateral cortex and hippocampus as a control. The lesion was approximately 1.5 cm from the pyramidal cell layer. Axotomized pyramidal tract neurons exhibited an early significant increase in [^{3}H]Act. D binding to nuclei at 1 day postoperation. This response had decreased rapidly to below normal levels ($p<0.05$) by 3 days postoperation. A second increase was noted at 5 days, but this response was not significantly above normal. This was followed by a sustained subnormal level of [^{3}H]Act. D binding from 7-11 days. During this time a gradual increase in binding occurred, until by day 14 levels did not differ from normal. The unoperated side of brain demonstrated only a transient decrease in [^{3}H]Act. D binding at 9 days postoperation relative to hippocampus.

Comparison of the two systems suggests in both cases a biphasic response in the pattern of chromatin changes. However, the central neuron reacts in a pattern indicative of severe injury with the second phase occurring rapidly (5 days) and transiently. The subsequent period of prolonged depression in [^{3}H]Act. D binding may be associated with cellular atrophy. The data indicates that some critical responses of the CNS cell nucleus occur within the first week after injury. This time period may be important for the implementation of therapeutic methods aimed at enhancing CNS regeneration.

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299.10 POSTTRANSLATIONAL PROTEIN MODIFICATION BY AMINO ACID ADDITION FOLLOWING INJURY TO RAT SCIATIC AND OPTIC NERVES. S.S. Athwal*, G. Chakraborty*, M.F. Zanakis and N.A. Ingoglia, (SPON: F.P.J. Diecke). Dept. of Physiology, New Jersey Medical School, Newark, N.J. 07103

Axoplasm obtained from squid giant axons, and axons of the rat sciatic and goldfish optic nerves are capable of post-translational protein modification (PTPM) by amino acid addition. This reaction increases dramatically during regeneration indicating that it may be involved in some aspect of the regenerative process.

The present investigation examines this reaction in a nerve which does not regenerate following injury, the rat optic nerve. The purpose of the study was to determine if the reaction occurs in this nerve and if so to compare the time course and magnitude of the reaction with that of the rat sciatic nerve.

Homogenates of normal optic nerves, like normal sciatic nerves, were able to incorporate low levels of ^{3}H-Arg, ^{3}H-Lys and ^{3}H-Leu into protein in a partially purified fraction of the 150,000 xg supernatant. In other experiments, nerves were crushed and proximal segments were assayed for posttranslational incorporation of amino acids into protein at 1, 3, 6 and 14 days following nerve crush injury. In regenerating rat sciatic nerves the magnitude of this reaction increases 2-3 fold 18 hrs after crush and reaches a maximum of between 10 and 25 fold 12-14 days later for each amino acid tested. Incorporation of amino acids into proteins in rat optic nerves was suppressed at 1 and 3 days following nerve crush (0.5 times normals) but increased to approximately 7.5 times normal 6 days after crush. However, by 14 days post crush preliminary data indicates that the reaction is again depressed (0.75 times normals).

The data indicate that in regenerating rat sciatic nerves PTPM by amino acid addition occurs throughout the time course examined in this study, but that in damaged (and not regenerating) optic nerves there is only a transient increase in the reaction. We speculate that in sciatic nerves these reactions are critical for the regrowth of damaged axons, but that in optic nerves the reaction is associated either with abortive sprouting of injured axons or with a non-neuronal (glial) reaction. Supported by NIH grant NS 19148.

299.11 POSTTRANSLATIONAL PROTEIN MODIFICATION BY POLYAMINES IN AXONS OF INVERTEBRATE AND VERTEBRATE NERVES. G. Chakraborty*, T. Leach*, M.F. Zanakis and N.A. Ingoglia. Department of Physiology, New Jersey Medical School, Newark, NJ 07103.

Protein modification by covalent addition of putrescine and spermidine occurs in the soma of the R2 neuron of Aplysia (Ambron and Kremzner, PNAS 79, 3442-3446, 1982). The current experiments were performed to determine if these reactions also occur in axons, and what effect nerve regeneration has on the magnitude of the reaction.

In the first series of experiments ^{3}H putrescine and ^{3}H spermidine were incubated with 150,000 xg supernatants obtained from brain, heart, liver, kidney and sciatic nerves of the rat. Levels of incorporation of radioactivity into a hot and cold TCA precipitable fraction were similar in all tissues (approx. 25-50 DPM/ug of protein), except in liver where the incorporation was on the order of 1000 DPM/ug of protein. In all cases the reaction was inhibited by 10mM $CuSO_4$, a potent inhibitor of transglutaminase, the enzyme required for the covalent addition of polyamines to proteins.

In other experiments, radioactive putrescine or spermidine were incubated along with the soluble supernatant fraction of axoplasm isolated from the giant axon of the squid. These extracts were also able to incorporate radioactivity into endogenous proteins (as well as N-N'-dimethylcasein added as an exogenous substrate), indicating that axoplasm contains the necessary elements for the posttranslational covalent modification of proteins by the addition of polyamines. These elements are also likely to be present in axons of vertebrate nerves since activity was found to build up proximal to a ligature applied to rat sciatic nerves. When these nerves were crushed 6 days prior to analysis, activity was increased 2 fold in regenerating shafts of the nerve, and approximately 3-4 fold in the most advanced portion of the growing nerve. Similar results have been obtained from experiments performed in regenerating optic nerves of goldfish.

These data suggest that axons contain the necessary elements for the modification of proteins by polyamine addition, and that this activity is increased significantly in nerves undergoing regeneration. Supported by NIH grant NS19148.

299.12 REGULATION AT THE mRNA LEVEL OF REGENERATION - ASSOCIATED CHANGES IN THE SYNTHETIC PRODUCTS OF NON-NEURONAL CELLS SURROUNDING GOLDFISH OPTIC NERVE. Rachailovich, I*., Stein-Izsak, C. and Schwartz, M. Dept of Neurobiology, The Weizmann Institute of Science, Renovot, Israel.

Crush injury of the goldfish optic nerve was shown to be accompanied by changes in the nature of proteins secreted by the surrounding non-neuronal cells. (Rachailovich & Schwartz, Brain Res, 1984, in press). The aim of the present work was to elucidate whether these changes are regulated at the RNA level. RNA was prepared from non-neuronal cells originating from regenerating and intact optic nerves. The RNA preparations were further purified by passing them through oligo-dT columns. The resulting poly-A$^+$fractions were then translated in reticulocyte lysate cell free systems. Translation was carried out in the presence of ^{35}S-methionine and human placental RNAase inhibitor. Electrophoretic analysis of the radiolabeled products revealed the presence of polypeptides having apparent molecular weights of 89kDa and 30kDa in the regenerating nerve preparation. The same polypeptides were hardly detectable in the translation products of the intact nerve. In addition, a polypeptide of 17kDa which appeared in the translation products of the regenerating prepartion could not be detected in the preparation of the intact nerve. A few polypeptides having apparent molecular weights of 44kDa, 39kDa and 20kDa were detected in the translation products of the intact nerve and were either absent or reduced in the regenerating nerve.

Interestingly, electrophoretic analysis of secreted molecules derived from both regenerating and intact nerves revealed the presence of 17kDa polypeptide only in regenerating nerve preparation. It is possible that this polypeptide and that encoded by the purified mRNA derived from regenerating nerve are related.

These results indicate that the regeneration - associated changes in the synthetic products of non-neuronal cells surrounding goldfish optic nerve are regulated at the mRNA level.

300.1 PHOTORECEPTOR DEGENERATION ASSOCIATED WITH MUTATIONS IN PRESUMPTIVE OPSIN STRUCTURAL GENE IN DROSOPHILA. D.S. Leonard* and W.L. Pak. Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.

A Drosophila locus has been identified that exhibits a gene dosage effect on functional rhodopsin content in only the major (R1-6) class of photoreceptors, indicating that this locus, ninaE, probably defines the structural gene for R1-6 opsin. Physiological studies of ninaE mutants isolated to date show that the functional R1-6 rhodopsin content is severely reduced, whereas R7 and R8 rhodopsin content is probably normal (Scavarda et al., 1983, PNAS 80:4441). To find out if these mutations have any effect upon photoreceptor cell structure, we have begun a systematic anatomical study of several ninaE mutants.

In four mutants examined thus far, rhabdomeres (organelles that contain most of the visual pigment) of R1-6, but not R7 or R8, photoreceptors degenerate as a function of age. Degeneration occurs with the same time course in the presence or absence of screening pigments and with or without exposure to light. We therefore conclude that R1-6 rhabdomere degeneration is light independent in these ninaE mutants, indicating that the degeneration is not triggered by some input from the phototransduction pathway.

Physiological data show that levels of R1-6 rhodopsin are severely reduced in different alleles of ninaE. There is approximately 10^{-3} as much R1-6 rhodopsin in ninaE^{P332}, and 10^{-6} as much in ninaE^{P334}, as in wild type (Johnson and Pak, 1983, Soc. Neurosci. Abstr. 9:683). ora^{JK84} contains two mutations, at ninaE and at another locus, but the mutation at the ninaE locus appears to be the only one that affects R1-6 rhabdomere structure (O'Tousa, Leonard and Pak, in preparation). In ora^{JK84}, intracellular recordings consistently yield no response (E.C. Johnson, personal communication). Anatomical data from these mutants indicate that degeneration is relatively slow in ninaE^{P332}, such that nine week old adults retain approximately 34% the normal number of R1-6 rhabdomeres. Degeneration is more rapid in ninaE^{P334}, such that R1-6 rhabdomeres are absent in nine week old adults. The most accelerated degeneration is seen in ora^{JK84}, where newly emerged adults have 90%, one week old flies 18%, and six week old adults essentially no R1-6 rhabdomeres. We conclude that the rate of R1-6 rhabdomere degeneration may be related to the functional severity of each mutation.

300.2 INDUCED ALTERATION IN THE DEVELOPMENT OF THE DROSOPHILA GIANT FIBER PATHWAY IN THE TEMPERATURE-SENSITIVE MUTANT SHIBIRE. M.R. Hummon* and W.J. Costello. Dept. Zool. and Biomed. Sci./Col. Osteo. Med., Ohio Univ., Athens, OH 45701

The mutant shibire can be used to generate specific alterations in the dorsolongitudinal flight muscles (DLM) (Costello and Salkoff (83) Neurosci. Abs. 9:832); here we describe an alteration in the giant fiber (GF) pathway to these muscles. In wildtype flies, the GF pathway mediates the escape response by activating the tergotrochanteral (jump) muscle (TTM) and the DLM's. The pathway to TTM (minimum latency, 0.8 msec) includes GF, with electrical synapses to TTM motoneuron (TTMn), which itself synapses onto TTM. The pathway to DLM (minimum latency, 1.2 msec) involves GF, with electrical synapses to the peripherally synapsing interneuron (PSI); PSI then makes chemical synapses onto the 5 DLM motoneurons (DLMn). The pathway to DLM therefore includes chemical synapses in the peripheral nerve (PSI-DLMn) and at the muscle.

Shibire flies were reared at the permissive temperature of 22 C; pupae were heat-pulsed at 30 C for 6 hr early in development. Such flies show fusion of the normal 6 DLM's on each side into 3 DLM's. Ganglion stimulation elicits a stepwise increase in EPSP's in the fused DLM's, implying that the DLM unit is innervated by two DLMn's normally innervating each component DLM. Direct stimulation to the brain also activates GF pathway components. Latencies to the TTM (0.92 $\pm$ 0.07 msec, n=9) are similar to that of wildtype, indicating that the GF-TTM pathway is intact and normal. However, minimum latencies to the DLM's (1.63 $\pm$ 0.16 msec, n=9) are longer than wildtype (1.2 msec). Synchrony of right and left DLM's is also lost.

Examination of heat-pulsed shibire flies with TEM shows that the GF is present. In the region of the peripheral nerve where TTMn, DLMn's, and PSI normally occur together, the TTMn and 5 DLMn's are present, but the PSI is absent. DLMn's make inappropriate synapses throughout the region, to glia, to space, and to poorly defined membranes. The PSI remnant can be found extending an abnormally short distance in the nerve; here it makes occasional synapses onto DLMn and inappropriate synapses onto glia and to space. Work is in progress to determine critical periods for development of components of the GF pathway.

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300.3 LONG-TERM POTENTIATION AND LONG-TERM ADAPTATION AT SYNAPSES OF A CRAYFISH PHASIC MOTONEURON. G.A. Lnenicka and H.L. Atwood, Department of Physiology, University of Toronto Toronto, Ontario, Canada M5S 1A8

We have previously shown that the normally depressible neuromuscular synapses of a crayfish phasic motoneuron (the "fast" excitor innervating the crayfish claw closer muscle) become more fatigue-resistant after in vivo tonic stimulation (Neurosci. Abstr. 9:53). Two weeks of 5 Hz stimulation for 2 hours/day produces a 44% decrease in amplitude of the initial EPSP, and a 4.3 fold increase in the final EPSP amplitude as tested during 30 minutes of 5 Hz stimulation of the fast axon. Adaptation results from changes in transmitter release, and persists for at least 10 days.

In order to examine the time course of the development of long-term adaptation (LTA), shorter chronic stimulation regimes were tested. EPSP measurements made 1 day after 2 hours of in vivo 5 Hz stimulation showed a 60% increase in the initial EPSP amplitude and no change in the final EPSP amplitude. This long-term potentiation (LTP) of the initial EPSP amplitude persists for at least 3 days. This stimulation regime produced no evidence of LTA.

After 3 days of in vivo stimulation of the fast axon at 5 Hz for 2 hours/day, LTA is evident. A 3.8-fold increase in the final EPSP amplitude was observed with no significant change in the initial EPSP amplitude. Thus, there was an increase in the ability of the terminals to maintain transmitter release during prolonged activation, and also, the initial transmitter output has apparently begun to decrease as evidenced by the lack of LTP.

To determine the role of the cell body in the establishment of LTP and LTA, the distal segments of sectioned fast axons were stimulated in vivo for 1 and 3 days at 5 Hz for 2 hours/day. One day of stimulation of decentralized axons produces LTP, indicating the cell body is not required for this effect. However, 3 days of stimulation did not produce the significant increase in the final EPSP amplitude normally associated with LTA. Thus, products from the cell body are apparently required for the establishment of LTA, while LTP is due to local changes at the synapses.

Experiments are currently in progress to determine whether the change in electrical activity centrally, or at the neuromuscular synapses is responsible for triggering the events leading to LTA.

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300.4 EFFECTS OF REGENERATION OF AUDITORY AFFERENTS ON DENDRITIC SPROUTING OF AN IDENTIFIED AUDITORY INTERNEURON. S.L. Pallas and R.R. Hoy. Neurobiology and Behavior Section Cornell University, Ithaca, NY 14853.

In the cricket Teleogryllus oceanicus, the arborizations of an identified auditory interneuron, Int-1, are normally restricted to the ipsilateral auditory neuropil; unilateral removal of the ear causes the medial dendritic field to sprout across the midline and make functional connections with the contralateral auditory neuropil (Hoy, Casaday and Rollins, Neurosci. Abstr. 4, 1978; Hoy and Moiseff, Neurosci. Abstr. 5, 1979). We have investigated the effects of regeneration of the auditory afferents on this aberrant contralateral dendritic projection of Int-1.

Crickets at various stages of postembryonic development were unilaterally deafferented by crushing the prothoracic leg nerve which contains the auditory afferents. The morphology of Int-1 was examined by cobalt backfilling. The backfills revealed sprouting across the midline in 76% (n=50) of all animals tested. No sprouting was observed in the other 12 animals. In contrast, in animals whose ear was removed or whose leg nerve was crushed repeatedly to prevent regeneration, contralateral sprouting was always observed. Functional regeneration of the afferents was tested with a behavior known to be mediated by Int-1, ultrasound avoidance (Nolen and Hoy, Neurosci. Abstr. 8, 1982). Deafferented crickets respond to ultrasound by turning away from the intact ear regardless of the location of the sound source. In this behavioral assay, 66% (n=86) of the animals whose leg nerve had been crushed once responded to ultrasound stimulation of both ears, indicating regeneration of afferents from the crush. Within this group, 31 animals were examined morphologically and sprouting was seen in 22 (71%). None of these results depended on the age of the animal at deafferentation.

Thus, interruption of the auditory afferents by crushing can cause contralateral sprouting in Int-1, and this abnormal projection is generally retained, despite regeneration of the crushed afferent fibers and formation of synaptic connections. In cases where sprouting was not seen, the regenerating afferents may have been able to prevent sprouting. Alternatively, the afferents may have caused a retraction of the contralateral dendrites after sprouting had been initiated.

300.5 EMBRYONIC DEVELOPMENT OF SEGMENTALLY SPECIALISED SEROTONERGIC NEURONES IN THE LEECH HIRUDO MEDICINALIS. A.J.R. Mason* J.C. Glover and W.B. Kristan, Jr. Dept. Biology, B-022, University of California at San Diego, La Jolla, CA 92093

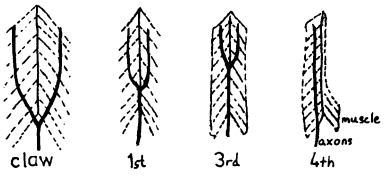
Most of the 21 segmental ganglia of the medicinal leech contain about 400 neuronal cell bodies and this set of neurones is repeated from ganglion to ganglion. However, body segments 5 and 6 contain the male and female reproductive structures and the ganglia innervating these segments are correspondingly specialised. Ganglia 5 and 6 contain about twice as many neurones and innervate the sex organs via additional segmental nerves. We wanted to know whether these specialised ganglia are different from the outset of embryonic development or whether they are initially identical to other ganglia and are later modified. We have studied the morphological development of a pair of large, serotonergic neurones (known as Retzius cells) present in all segmental ganglia. In most ganglia of the adult leech, each Retzius cell sends axons to the periphery and also to the adjacent segmental ganglia via the connectives. In ganglia 5 and 6 the Retzius cells lack axons in the connectives but are otherwise similar. Using a serotonin antibody and intracellular Lucifer Yellow injections we have shown that all segmental ganglion Retzius cells have the same morphology in young embryos. During later development, however, the connective branches of the Retzius cells in ganglia 5 and 6 fail to elongate as fast as the ganglia and connectives increase in size. This results in the connective axons extending less and less far into the connectives. Eventually, the axons do not exit the ganglion and cannot be distinguished from Retzius cell dendrites within the neuropil.

What causes the difference in Retzius cell connective axon growth between ganglia 5 and 6 and the other ganglia? The explanation may be related to differences in peripheral innervation targets of the Retzius cells. The peripheral axons of most Retzius cells branch repeatedly in the body wall of an entire hemi-segment, probably to innervate muscles and mucous glands. However, the Retzius cells of ganglia 5 and 6 innervate reproductive structures. Since other Retzius cells do not innervate these structures, it is possible that contact between the peripheral axons and reproductive tissue during development signals the Retzius cells of ganglia 5 and 6 to stop extending their connective axons. We are currently investigating this possibility.

This research was supported by grants from NSF and the March of Dimes.

300.6 AXONAL BRANCHING TO THE OPENER MUSCLE IN LOBSTER CLAWS AND WALKING LEGS. K.M. MEAROW and C.K. GOVIND. Scarborough College, Univ. of Toronto, West Hill, Ontario, Canada.

The opener muscle of the claws and the four walking legs in the lobster, Homarus americanus, is innervated by a single excitor and an inhibitor motor neuron (although it is possible that additional inhibitory innervation is present). We have been investigating the following characteristics of the opener muscle in these serially homologous structures: i) the major branching pattern of the excitor and inhibitor axons using methylene blue staining; ii) the gross morphology of the muscle using histochemical techniques; and iii) the electrophysiological characteristics of the neuromuscular junctions using conventional recording techniques. In the claws and the first 3 walking legs, the axons bifurcate providing a major branch to each side of the bipinnate muscle. The location of this major bifurcation varies, being situated proximally on the muscle in the claws and distally in the 3rd walking leg (Figure). In the 4th walking leg, however, there is no major bifurcation; in addition, the muscle is not symmetrically arranged about a central tendon in a bipinnate fashion. The axons travel along the side of the muscle displaying the most fibres; examination of cross-sections of the muscle show that the fibres have rotated under the tendon to insert on one side only. The above correlation seen between the presence or absence of a major axon bifurcation with the bipinnate or pinnate arrangement of the muscle reflects some trophic interaction between neuron and muscle in these homologous structures. This is further supported by aberrant cases such as a major axonal bifurcation in a 4th walking leg where the muscle was symmetrically arranged, and no axonal bifurcation in a 3rd walking leg where the muscle was asymmetric. Experiments manipulating either the axon or the muscle during development or regeneration may demonstrate the interdependence between the axonal branching pattern and muscle fibre arrangement. Supported by NSERCC and MDAC.



SPECIFICITY OF SYNAPTIC CONNECTIONS

301.1 THE MORPHOLOGY OF ISTHMO-TECTAL AXON ARBORS IN DEVELOPING XENOPUS FROGS. Susan B. Udin. Div. Neurobiology, Dept. Physiology, SUNY, Buffalo, NY 14214

The nucleus isthmi (NI) of the amphibian relays visual input from one tectum to the other tectum and thus brings a visual map from each eye to its ipsilateral tectum. The ipsilateral map develops in register with the contralateral retinotectal map if normal visual experience is allowed.

In this abstract, I report on the morphology of normal isthmo-tectal axon arbors at different stages of development. Axons were filled by anterograde transport of HRP from the nucleus isthmi. The tecta were reacted as flat-mounts (S.B. Udin and M.D. Fisher, 1983, J.Neurosci.Meth. 9:283).

Stages 57-59: The eyes are laterally-directed and there is no binocular field. Nevertheless, isthmo-tectal axons do enter the tectum at these stages. The axons can extend over at least 80% of the extent of the tectum. The axons are very sparsely branched.

Stages 60-66 (metamorphic climax): The eyes begin to migrate dorsally; binocular overlap develops; and isthmo-tectal units can be recorded in rostral tectum (S.Grant and M.J.Keating, 1981, J.Physiol. 320:18P). Isthmo-tectal arbors begin to arborize profusely. These arbors first are confined to the very rostral margin of the tectum; but they subsequently extend more caudally. The arbors are larger than in mature adults, and there often are long branches which extend away from the main arbor.

Juveniles (2-8 weeks post-metamorphosis): Eye migration continues at a slower rate. Many axons retain an immature appearance, with wide-spread arbors plus scattered branches, while other axons appear much more like adult axons, with compact arbors and few or no extraneous branches.

These patterns are consistent with the hypothesis that axons can enter "inappropriate" tectal regions (e.g., all of the tectum prior to stage 60 or caudal-most tectum at later stages) but that the axons only form relatively dense arbors in the binocular regions, where their visual fields can match the visual fields relayed from the opposite retina (e.g., the rostral tectal margin at stage 60-62). The many "extraneous" branches may serve to "test" regions of the tectum and allow the arbors to shift caudally as the binocular segment of the tectum expands caudally.

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301.2 THE SPECIFICITY OF INNERVATION AMONG XENOPUS TWITCH MUSCLE FIBERS. B. M. Nudell and A. D. Grinnell. The Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles, CA 90024.

In an earlier paper we reported that over 50% of the Xenopus pectoralis muscle fibers with two distant endplates were innervated at both sites by branches of the same motor neuron (mononeuronal innervation). It seemed unlikely that random innervation of muscle fibers would result in so much mononeuronal innervation, since it has been reported that 50 motor neurons innervate this muscle. In order to determine whether some selective influences underlie this ordered pattern, we have extended our study to examine carefully the innervation pattern of individual motor neurons within the pectoralis muscle. We find that the muscle fibers receiving suprathreshold innervation from a given motor neuron are of similar size (input resistance), and that three distinct classes of motor units can be distinguished on the basis of fiber size. While all three classes of motor unit are represented in each section of the muscle, individual motor units are spatially localized within the muscle and similar type units are largely segregated from one another. Overlap between motor units of the same class seems to occur primarily at their common borders. These studies also confirm that there is a high incidence of mononeuronal innervation in the pectoralis muscle. Among the large muscle fibers which comprise one class of motor unit for instance, the incidence of mononeuronal innervation is 66%. On the basis of these studies, we suggest that the spatial segregation of the synaptic projection fields of motor neurons innervating similar sized muscle fibers may be an important factor in generating the high incidence of mononeuronal innervation.

301.3 THE ANATOMICAL RELATIONSHIP BETWEEN SPINDLE AFFERENTS AND MOTONEURONS DURING SYNAPTOGENESIS

P.C. Jackson and E. Frank, Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60201.

Monosynaptic inputs from muscle spindle afferents onto brachial motoneurons are as specific when first seen electrophysiologically in the tadpole (Stage XVII) as they are in the mature frog (Frank & Westerfield, J. Physiol. 343:593, 1983). From the outset triceps spindle inputs to triceps motoneurons are stronger than those to other classes of motoneurons. However, if the sensory and motor cells were in anatomical proximity well before functional synapses could be detected, synaptic specificity might result from re-arrangements of physiologically undetectable synaptic contacts. The present work was undertaken to examine when triceps sensory axons and triceps motoneurons first come into anatomical proximity with each other.

Populations of triceps sensory and motoneurons were labelled in tadpoles (Stages XIV-XIX) by backfilling the triceps nerve in vivo with HRP. In other tadpoles hemisected spinal cords were removed from the animal and individual triceps motoneurons were impaled with micro-electrodes and filled with HRP.

By Stage XIV triceps sensory afferents projected to and arborized in the region of the spinal cord where sensory-motor connections are eventually made. In contrast, the dendrites of triceps motoneurons rarely were present in this region until late in Stage XVI. The very fine branches of dendrites projecting more laterally, medially, and ventrally were well filled, so the paucity of dendrites seen in the dorso-medial region of future monosynaptic connections is unlikely to be ascribable to poor filling of the cells. At mid to late Stage XVII, dorso-medial dendrites were present and intermingled with the collaterals of muscle sensory axons. Thus, the sensory axons grow into the future neuropil region several stages prior to the arrival of motoneuronal dendrites. Further, the time when the axons and dendrites come into close proximity correlates well with the time that monosynaptic connections are first detected between these two populations.

301.4 PROJECTIONS OF SENSORY NEURONS IN TRANSPLANTED DORSAL ROOT GANGLIA

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During development, sensory neurons innervate structures in the periphery and establish synaptic connections with the appropriate groups of neurons in the central nervous system. We are examining both the peripheral and central projections of neurons in dorsal root ganglia (DRGs) transplanted to a different level of the neuraxis to determine whether the connections formed by these neurons are influenced by their novel environment.

Transplantations are performed in Rana catesbeiana tadpoles at Stages IX-XIV, which includes the period during which muscles and skin of the forelimb are innervated. DRG 2, which normally innervates the forelimb, is removed and replaced with both DRGs 4 and 5. In the normal adult frog, DRGs 4 and 5 are composed mainly of cutaneous afferents that innervate skin of the trunk. After the animals metamorphose, their responses to mechanical stimulation of the forelimb are examined. Then, the projections of sensory neurons in the transplanted ganglia are traced by cutting the 2nd spinal nerve, which innervates the forelimb, and exposing the central stump to HRP.

Sensory neurons in the transplanted ganglia innervate the forelimb and form functional synaptic connections in the brachial spinal cord. Anatomically, the central projection resembles that of the normal DRG 2 in that it consists of two distinct plexuses, one located in the dorsal horn and the other in the intermediate gray matter. Afferents contributing to the latter plexus have varicosities that appear to contact the dendrites of brachial motoneurons. This projection is characteristic of muscle afferents and is not present in the thoracic spinal cord. Experiments now in progress are designed to determine whether the sensory neurons in the transplanted ganglia that arborize in the more ventral plexus in the spinal cord are muscle afferents and whether they innervated trunk skin prior to their transplantation.

301.5 PATTERNED ACTIVITY IS NOT CRITICAL FOR THE DEVELOPMENT OF SPECIFIC SENSORY-MOTOR SYNAPSES IN THE SPINAL CORD

E. Frank and P.C. Jackson, Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60201.

In many developing systems, neural activity is important in determining the specific patterns of synaptic connections among neurons. The experiments described here were designed to explore the effect of patterned activity on the development of specific synaptic connections between stretch-sensitive muscle afferent axons and motoneurons in the brachial spinal cord of the bullfrog.

Triceps muscle sensory axons provide strong, excitatory monosynaptic input to triceps motoneurons, but project only weakly to subscapularis and pectoralis motoneurons. We used this differential pattern of sensory projections to test the effect of altering the neuronal activity in a subset of the triceps sensory cells. The medial triceps tendon was cut in Stage XIV tadpoles, several stages before the onset of synaptogenesis between these sensory and motor cells in the spinal cord. The tendons of the synergistic internal and external triceps muscles were left intact. Tenotomy causes a drastic reduction in the discharge of muscle spindles by eliminating the ability of elbow flexion to stretch the triceps muscle. The consequences of reduced sensory activity were assessed after metamorphosis by making intracellular recordings from motorneurons and measuring the input these neurons received from sensory axons innervating the tenotomized and normal triceps muscles.

The monosynaptic connections made were normal; medial and internal-external triceps sensory fibers projected to both classes of triceps motoneurons more strongly than to non-triceps motoneurons, just as in normal frogs.

In several Stage XX tadpoles (metamorphic climax) the medial triceps tendon was cut and re-inserted on the medial surface of the radio-ulnar bone just below the elbow such that the muscle was stretched by elbow extension rather than by elbow flexion. Even in these cases when the medial and internal-external triceps muscles had been made functional antagonists, sensory-motor connections were normal. Therefore, major disruptions of the normal pattern of muscle sensory activity seem not to disrupt the development of normal sensory-motor connections. Neural activity apparently does not play a decisive role in the establishment of specific synaptic connections between muscle afferent fibers and motoneurons.

301.6 FORMATION OF TARGET-SPECIFIC AXON TERMINAL DISTRIBUTIONS IN EMBRYONIC NIGRO-STRIATAL DOUBLE GRAFTS.

C. B. Jaeger, Depts. Pharm. & Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Neural transplants placed into the brain of a suitable host readily become vascularized and often show the cyto-architectonic characteristics of their origin. Therefore, neural grafts permit studies of the developmental potential of isolated neuron groups. This investigation, examined specific cell interactions and their possible influence on the differentiation and survival of grafted dopaminergic (DA) neurons and associated glia. Glia filament proteins (GF) of astroglia and tyrosine hydroxylase (TH) of DA neurons were localized in the grafts by immuno-cytochemical procedures. Synaptic contacts of labeled DA neurons in grafts where revealed by electron microscopy. Donor tissue of rat embryos (E 14-16) was taken from the ganglionic eminence, ventral mesencephalon, or both and placed either superficial or deep into cerebral cortex, tectum, cerebellum, or ventricles of newborn rats. Transplants persisted in the different host brain regions with the exception of single nigral grafts in cerebellar white matter. There grafts degenerated within two weeks of transplantation. Nigral grafts commonly contained groups of DA neurons that formed arching dendritic bundles characteristic of substantia nigra compacta. Activated astroglia associated with DA neurons. Some superficial grafts connected to the host brain by a tissue stalk. Such stalks consisted of parallel astroglia and projection fibers including TH positive axons. Labeled terminals in nigral grafts distributed diffusely and synapsed predominantly on dendrites. A striking change of DA neuron differentiation occurred in grafts co-transplanted with striatal tissue. In such transplants the TH positive fibers derived from DA neurons formed dense patches of terminals co-extensive with grafted striatal neurons. In contrast, nigro/cerebellar co-grafts exhibited ramified DA fibers, but lacked dense axon terminal clusters. These studies indicated that differentiation of DA neurons may be significantly affected by specific cell interactions. The associations of glia and DA neurons emphasize the role of astroglia in the establishment of cytoarchitectonic structure. Supported by NIH Grant NS19699.

301.7 HOMOTYPIC GRAFTS OF ENTORHINAL CORTEX: CONNECTIONS WITH THE HOST BRAIN. R.B. Gibbs, E.W. Harris and C.W. Cotman. Dept. of Psychobiology, U.C. Irvine, Irvine, Ca. 92717.

Embryonic entorhinal cortex was implanted into the entorhinal region of thirty young adult rats (wt. 150-200g) which had received a lesion through the angular bundle ten days previously. One to six months after implantation, connectivity between the implants and host brains was examined using fast blue and wheat germ agglutin-horseradish peroxidase (WGA-HRP). Adjacent sections were stained for the presence of acetylcholinesterase (AChE) and with cresyl violet. Our goal was to determine if damaged cortical projections could be replaced by implants of an homologous cortical area.

Implants of embryonic entorhinal cortex selectively reinnervated regions of the host hippocampus and amygdala which normally receive entorhinal input. Implant fibers which innervated the hippocampus were restricted to the stratum lacunosum moleculare of CA1 and to the molecular layer of the dentate gyrus. Implant fibers were also observed within the lateral amygdaloid nucleus and in the baso-lateral amygdaloid nucleus. In animals which had not received a knife cut through the angular bundle, fewer implant cells projected to the host hippocampus suggesting that outgrouwth from the implant is facilitated by denervation of the host. Implant projections were not observed within the lateral posterior nucleus of the thalamus, the fimbria or in adjacent cortical areas.

Implants received projections from the host septum/diagonal band. After injecting WGA-HRP into the host septum, the distribution of HRP labelled fibers within the implant matched the distribution of AChE positive fibers observed within adjacent sections. Unlike the projections from implant to host, host septal fibers strongly innervated the implants in the absence of a knife cut through the angular bundle. Implants failed to receive projections from the host amygdala, pyriform cortex, hippocampus or other cortical areas, except in one animal where the implant contained fibers from the contralateral entorhinal cortex. The distribution of these fibers coincided with the distribution of septal fibers observed within adjacent sections.

In conclusion, implants of embryonic entorhinal cortex reinnervate specific areas of the host brain denervated by a lesion through the angular bundle. Functional aspects of these connections are currently being studied.

(Supported by NIMH grant no. MH19691)

301.8 THALAMIC PROJECTIONS TO EMBRYONIC FRONTAL CORTEX TRANSPLANTED * INTO NEWBORN RAT CORTEX. F.-L.F. Chang, J.G. Steedman *, and R.D. Lund. Dept. of Anatomy & Cell Biology, Center for Neuroscience, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

In a previous study (Develop. Brain Res., 13:164) we transplanted embryonic occipital cortex into the parieto-occipital (P-O) cortex of newborn rats and found that the thalamic nuclei projecting to such transplants are those which normally project to the host cortical area surrounding the transplant. In the present study, embryonic frontal cortex was transplanted to the same P-O region to examine whether the source of the transplant (occipital vs. frontal) affects the projection pattern from the host thalamic nuclei.

Pieces of frontal cortex (1-2mm^2) from E15 rat embryos were dissected out in F10 medium (Gibco). Host rats were anaesthetized with ice, a shallow cavity in the left P-O cortex was created by suction, and the transplant tissue was transferred to it with a fine hair loop. The procedures matched those in the previous study. After one month survival, neural connections were traced by depositing horseradish peroxidase (HRP) in the transplant either as a dried pellet on the tip of a fine needle, or by iontophoretic injection of a 10% solution through a glass micropipet (tip diameter 10 µm, 1 µA for 20 min.). After 24h survival time, brains were sectioned frozen at 40 µ m and reacted with tetramethylbenzidine.

Retrogradely labeled cells were examined in the host thalamus. Thalamic nuclei that projected to frontal cortical transplants included ventrolateral (VL), latero-posterior (LP), laterodorsal (LD), and posterior (PO). These nuclei also project to occipital cortex transplants. In two cases we intentionally made a large HRP deposit to include the whole extent of the transplant, but we could not find retrogradely labeled cells in the ventroanterior (VA) or dorsomedial (DM) nuclei which normally project to the frontal cortex. We conclude that cortical tissue at E15 is unable to attract the ingrowth of thalamic afferents specific to its position of origin in the cortex. Rather, the afferents to a transplant seem to be dictated by its location in the host; nuclei which normally project to the surrounding cortex now project into the transplant. Transplantation of frontal cortex into frontal cortical host regions is presently under investigation.

Supported by NIH grant EY03326 to R.D.L.

301.9 EARLY DEVELOPMENT OF PROJECTIONS FROM EMBRYONIC RETINA TRANSPLANTED INTO THE HOST BRAIN OF RATS. L.K. McLoon and S.C. McLoon. Depts. of Ophthal. and Anat., Univ. of Minn., Minneapolis,MN 55455.

Fetal retinae transplanted adjacent to the superior colliculi of newborn rats differentiate and develop axonal projections into the host brain. One month after transplantation, the transplants project only to nuclei of the host brain which normally receive retinal connections. The question arises as to how the specificity of these projections from the transplant to host brain develop. Since other neuronal tissue transplanted to the same location in the same manner have a completely different projection pattern, it seems unlikely that simple mechanical cues are responsible for the pattern of connections formed by retinal transplants. Another possibility is that early projections from the transplants are somewhat random, and then by a process of pruning or cell death only axons with connections to visual nuclei are retained. To test this we have studied the projections of retinal transplants during their early stages of development. Retinae from rat embryos on the fourteenth day of gestation were dissected and cultured overnight in tissue culture media (supplemented MEM) containing various mixtures of tritiated amino acids (proline or leucine) or a fluorescent dye (True Blue, Fast Blue or rhodamine isothiocyanate). The retinae were then washed in tissue culture medium and transplanted to the superior colliculus of newborn rats. At one day intervals starting with the third day and up to twenty days after transplantation, the rats were perfused and the brains processed for autoradiography or fluorescence microscopy. As early as three days post-transplantation labelled projections were seen from the transplants. These axons ran along the surface of the host colliculus. By the fourth day, axons reached as far forward as the lateral geniculate nucleus. At each of the visual nuclei the axons left the brain surface and appeared to project into the nucleus. There was no suggestion of widespread projections at any time during the early developmental times examined. Thus, it would appear that the initial outgrowth from retina transplanted to newborn superior colliculus is specific and goes only to those visual nuclei with which it will subsequently connect.

(Supported by NIH grants EY05371 and EY05372).

301.10 TOPOGRAPHIC SPECIFICITY OF THE ABERRANT CORTICORUBRAL PROJECTION FOLLOWING NEONATAL CORTICAL LESIONS IN THE RAT. C.G. Naus*, B.A. Flumerfelt and A.W. Hrycyshyn*. Department of Anatomy, The University of Western Ontario, London, Canada.

The corticorubral projection was studied in neonatally hemispherectomized rats using anterograde transport of horseradish peroxidase conjugated to wheat germ agglutinin (HRP-WGA). The right cerebral cortex of neonatal rats was ablated and 6-8 weeks later the left cerebral cortex was injected with 1-2 µL of 1% HRP-WGA. The results were compared with those following similar injections in unlesioned rats.

In control animals, dense bundles of labeled fibers of the reciprocal corticothalamic projection could be followed through the caudate putamen and globus pallidus to the ipsilateral thalamus. Some labeled fibers appeared to filter through the thalamus to the midbrain. In addition, many labeled fibers coursed from the cortex through the internal capsule and cerebral peduncle. Corticorubral fibers were seen leaving this corticofugal projection to terminate ipsilaterally in the rostral two-thirds of the red nucleus. Other areas of labeling included the ipsilateral zona incerta, nucleus parafascicularis prerubralis, superior colliculus and midbrain reticular formation, as well as the corticospinal tract.

In neonatally lesioned adults, the unlesioned cortex gave rise to a bilateral corticorubral projection. The aberrant corticorubral fibers crossed the midline in the dorsal tegmental decussation and terminated contralaterally in the rostral two-thirds of the red nucleus. Terminal labeling in the contralateral red nucleus was less dense than the ipsilateral rubral labeling. Aberrant crossed projections were also observed in the contalateral thalamus, nucleus parafascicularis prerubralis, superior colliculus and midbrain reticular formation. Labeled corticospinal fibers were followed caudally to the pyramidal decussation, where they could be seen projecting bilaterally to the dorsal funiculi.

Unilateral ablation of the cerebral cortex resulted in a bilateral corticorubral projection and the topographic specificity was maintained in the aberrant contralateral input.

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301.11 SYNAPSES FORMED BY SINGLE AFFERENT AXONS FROM MEDIAL HABENULA TO INTERPEDUNCULAR NUCLEUS IN RATS. N.J. Lenn and L. Whitmore, Department of Neurology and Clinical Neurosci. Res. Center, Univ. of Virginia, Charlottesville, VA 22908.

Axons from the medial habenula (MH) enter the interpeduncular nucleus (IPN) rostrolaterally. Separate portions of MH project to separate subnuclei of IPN where they form different types of endings. Existing data based on random sections suggest that one subgroup of axons forms the well known spiral pattern with multiple recrossings of the intermediate and central subnuclei of IPN. These would therefore be expected to form many synapses, including one-half of crest synapses in both intermediate subnuclei and S synapses in the central subnucleus. These hypotheses were directly tested by EM of serial sections after stereotaxic injection of HRP into one fasciculus retroflexus (FR). Individual axons were followed through up to 120 sections. It was found that axons forming crest synapses are irregular in diameter and wavy in trajectory in the horizontal plane. Within one intermediate subnucleus they have only been seen to form one crest synapses and up to three symmetrical contacts. While some of these latter appear to be synapses, others may be non-synaptic. These axons are synapse free through long distances relative to the width of the intermediate subnuclei. Some of these axons enter dendritic glomeruli, form only one crest synapse, and leave without branching. There is no evidence that two branches of one axon contact both sides of a crest synapse. Axons from one FR do form crest synapses in both intermediate subnuclei. In a few cases both endings at a crest synapse are filled with HRP, but the remainder have only one ending filled. The occasional crest synapses with a myelin sheath contacting one side are not different in any other way, and may represent a very rapid response to the trauma of the injection. It is anticipated that additional features will be clarified as axons are followed over greater distances. (Supported by NIH Grant #NS 16882).

301.12 INAPPROPRIATE SYNAPSE FORMATION BY MISDIRECTED REGENERATING OPTIC FIBERS IN GOLDFISH: AN ELECTRON MICROSCOPIC HORSERADISH PEROXIDASE STUDY. W.P. Hayes* and R.L. Meyer (SPON: M. Bronner-Fraser). Developmental Biology Center, University of California, Irvine 92717.

A recent strategy for the study of the formation of selective axonal connections has been the deflection of selected optic fibers from a donor optic tectum(OT)into a host OT denervated by contralateral eye enucleation. Optic fibers from the medial or lateral brachia(MB or LB) were rerouted into the anteromedial region of the host OT. Previous autoradiography showed that deflected MB fibers projected medially whereas the deflected LB fibers projected into the lateral region of the host OT. However the misdirected LB fibers exhibited significant medial label as well(Meyer, 1984, J.Neuroscience 4:234). To distinguish whether this retinotopically inappropriate label represents fibers of passage or synaptic contacts this ultrastructural analysis was undertaken.

In order to identify optic fiber terminations horseradish peroxidase(HRP)was applied to the cut optic nerve and tectal sections were processed using the diaminobenzidine-cobalt protocol for electron microscopy. In normal fish and in fish with optic nerve crush(30-150 days post-crush)retinal fibers were uniformly labeled along the anteroposterior and mediolateral extent of the OT. Terminations were observed in three tectal lamina: the Superficial Fiber and Gray(SFGS), Central Gray and the deepest Central White(SAC). Except for synaptic vesicles and mitochondria which tended to exclude label, optic terminals were entirely filled with product and there was little to no evidence of terminal degeneration.

In fish with deflected LB fibers many HRP-filled terminals were observed bearing numerous asymmetric synaptic contacts in the SFGS of the inappropriate anteromedial host OT. A few labeled non-retinotopic terminals were also seen in the deeper SAC. These synaptic terminals were morphologically similar to normal and non-misdirected regenerating terminals, although clustering of numerous labeled terminals usually apparent was less evident. Heavily labeled deflected optic fibers formed large fascicles of myelinated axons in the optic fiber layer and in the SFGS.

Extracellular recordings on fish with LB deflections confirmed that only LB fibers were present in the medial host OT. Interestingly, the projection on the medial half of the host OT was found to be consistently reversed along the mediolateral axis so that the extreme lateral fibers were represented most medially on the host OT.

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301.13 SYNAPTIC PLASTICITY IN CULTURE: SWITCH FROM CHEMICAL TO ELECTRICAL CONNECTIVITY BETWEEN CULTURED APLYSIA NEURONS. Rolf Bodmer* and Irwin B. Levitan. Friedrich Miescher Inst., Basel, Switzerland and Biochem. Dept., Brandeis Univ., Waltham, MA 02254.

Adult Aplysia neurons maintained in primary culture can regenerate neurites which interconnect in elaborate networks. Under culture conditions which we have described previously (Dagan & Levitan, J. Neurosci. 1(1981)736-740), electrotonic connections between pairs of cells form with high frequency, but chemical synapses are observed only rarely. One pair of unidentified neurons from the abdominal ganglion exhibited synaptic plasticity in culture. At the start of recording from this pair of cells (after 5 weeks in culture) they showed no evidence of being electrotonically connected, but there was a chemical synapse between them. This synapse was unidirectional and was blocked reversibly by removal of calcium from the extracellular medium. Hyperpolarization of the postsynaptic cell revealed that the synaptic response consisted of a fast and a slow component. Voltage clamp analysis indicated that the fast component was most likely due to a decrease in a resting outward current. The slow component was probably due to an increase in outward current, but a decrease in a voltage-dependent inward current could not be ruled out. Pharmacological experiments implicated serotonin and acetylcholine as possible neurotransmitters for the slow component. These experiments were carried out over a period of three days, during which time the culture dish was perfused with physiological saline containing glucose, in place of the nutrient-rich serum-supplemented L-15 culture medium in which the cells had been grown for the previous five weeks. By the third day of perfusion the chemical synapse could no longer be detected, and the cells were electrotonically connected (coupling coefficient = 0.2). Thus adult neurons can exhibit plasticity in their choice of type of synaptic connectivity, and this choice can be profoundly influenced by environmental factors.

302.1 SENSITIVITIES TO NOREPINEPHRINE AND SEROTONIN IN DEVELOPING DORSAL RAPHE NEURONS. D.A. Smith, J.F. Bates,* and D.W. Gallager, Dept. of Psychiatry and Neuroanatomy, Yale Univ. School of Med., New Haven, CT 06508.

Electrophysiological and quantitative, autoradiographic receptor binding techniques were used to characterize the postnatal development of α_1- norepinephine (NE) and 5HT receptors in the midbrain dorsal raphe (DR) nucleus.

Dose-response curves for NE sensitivity were obtained by superfusing midbrain slices which included the midbrain raphe from rats of various postnatal ages with artificial CSF containing increasing doses (0.5 to 15 µM) of the α_1-NE agonist, phenylephrine (PE). DR cells recorded in the slice preparation were found to be sensitive to PE at all ages tested. There was no consistent relationship between the postnatal age of the rat and the sensitivity of DR neurons to PE (half-maximal excitatory dose of PE was 2.4 ±1.2µM). This electrophysiological data is consistent with autoradiographic evidence for α_1-adrenergic binding sites (as identified by specific ^{3}H-Prazosin binding) in rats at all postnatal ages tested. When measured with 0.8nM of ^{3}H-PZ added, sites were 18.8, 22.6, 22.6, 22.6, 24.9 fmoles/mg tissue at 1,3,5 & 7 days postnatal age and in adults respectively.

Sensitivity to LSD, used as a measure of 5HT agonist activity without affinity for the 5HT uptake system, was also tested in midbrain slices from rats of various postnatal ages. Preliminary results show that doses of LSD required to inhibit firing of DR neurons decrease with increasing age. These data suggest that sensitivity to LSD increases with age (for example, 3-day LSD IC_{50} = 400nM vs adult LSD IC_{50} = 140nM). These data are consistent with autoradiographic evidence for progressive increases in 5HT binding sites (as identified by specific ^{3}H-5HT binding) with increasing age. ^{3}H-5HT sites (with 8nM ^{3}H-5HT added) were found to be present at 1-day postnatal age (0.64 fmole/mg tissue) and increased during maturation (1.38, 2.16, 3.44, 6.73 and 10.43 fmoles/mg tissue at 3,5,7,14 and 21 days postnatal age respectively).

These data suggest the dorsal raphe nucleus exhibits pharmacologically and functionally mature responses to NE by postnatal day 1. However, progressive increases in both functional 5HT responsitivity and 5HT binding sites in the DR suggest that the 5HT receptor system matures during the early postnatal period. (Supported by: Klingenstein Foundation, USPHS NS 19655, State of Connecticut and Oberlin College).

302.2 HIGH-AND-LOW [^{3}H]IMIPRAMINE BINDING SITES IN DEVELOPING LONG EVANS RAT BRAIN. R. Sircar, J.R. Teni, S.R. Zukin, and H.M. van Praag*, Dept. of Psychiatry, Albert Einstein College of Medicine, Bronx, NY 10461.

High- and low-affinity [^{3}H]imipramine binding sites have been reported in cortex of adult mice and rat (Reith et al. 1983; Conway and Brunswick, 1983). Mocchetti et al. (1982) reported that high-affinity [^{3}H]imipramine binding sites develop about day 5 in rat pups and that by 12 days of age the numbers of binding sites reach adult values. They further noted that although no high-affinity [^{3}H]serotonin uptake was seen in brain until day 5, low-affinity [^{3}H]serotonin uptake was present on day 3. The present study was aimed at further analysis of the ontogeny of high-affinity [^{3}H]imipramine binding in rat brain and also to examine the potential differential development of high- and low-affinity [^{3}H]imipramine binding sites.

Brains from 3,6,9,12 and 15 day old Long Evans hooded rat pups were homogenized in 50 mM Tris-HCl containing 120 mM NaCl and 5 mM KCl (pH 7.5 at 7°C). 2-6 brains were pooled to give a final protein concentration of about 0.5-1 mg/ml. Adult brains were assayed as controls. 500 µl aliquots of homogenate were incubated (1 hr at 4°C) with 0.1 to 500 nM [^{3}H]imipramine, in the presence and absence of 100 µM desimpramine. Binding was terminated by rapid filtration under reduced pressure through GF/B filters. Data analysis was performed using the LIGAND program (Munson, 1979; Teicher, 1982).

Non-linear regression analysis showed the presence of a single saturable binding site in 3 day old rats, having an apparent Kd of 12.2 nM and B_{max} of 658 fmol/mg protein. In 15 day old rat brain a good 2-site fit was seen; an apparent high-affinity site with a Kd of 8.15 nM and B_{max} of 396 fmol/mg protein and a low-affinity site with a Kd of 178 nM and B_{max} of 2.37 pmol/mg protein. These values differed from those obtained in the case of the adult brain where the apparent Kd for the high- and low-affinity sites were 7.0 nM and 692 nM and B_{max} values were 455 fmol/mg protein and 8.68 pmol/mg protein respectively. High- and low-affinity sites were also detected in 12 day old rat brain. A saturable low-affinity site made its appearance between 6-9 days after birth.

The differential appearance of the apparent high- and low-affinity [^{3}H]imipramine binding sites suggests that they may have differing functional roles.

302.3 ONTOGENETIC CHANGES IN PINEAL GLAND MELATONIN SYNTHESIS IN VITRO. P.L. Garvey*, K.A. Haak*, T. Swink*, R.L. Terry, and L.D. Lytle. Laboratory of Psychopharmacology, Department of Psychology, Univ. of California, Santa Barbara, CA 93106.

In adult animals, synthesis of the pineal gland hormone, melatonin (MEL), depends in part on the release of norepinephrine from postganglionic sympathetic neurons onto pinealocyte β-noradrenoceptors. These changes in MEL synthesis are controlled by a receptor-linked mechanism which induces activity in N-acetyltransferase, an enzyme which rate-limits an intermediate step in the synthesis of MEL from its ultimate precursor amino acid, l-tryptophan. Characterizations of possible developmental changes in MEL synthesis have been carried out previously under in vivo conditions. Unfortunately, several artifacts [possible maternal-to-fetus and maternal-to-neonate transfer of MEL molecules; aging differences in hepatic MEL catabolism (see D.C. Klein et al., Life Sci. 28: 1975 (1981) or A. Altar, Dev. Neurosci. 5: 166 (1982) for reviews)] may have interfered with accurate assessments of these maturational changes.

We have reassessed possible maturational changes in pineal gland MEL synthesis under in vitro conditions to eliminate some of these potential artifacts. Pineal glands obtained from fetal (postconception day 19), neonatal (0 days old), or adult (50 days old) albino rats were incubated in vitro in the presence of a 10^{-3} N HCl vehicle or with the β-noradrenoceptor agonist drug isoproterenol (10^{-4} M) for 4 hr. Changes in pineal gland or culture media concentrations of precursor [tryptophan (TRP), 5-hydroxytryptamine (5HT), N-acetylserotonin (NAS)] or end-product (MEL) compounds were measured using the high performance liquid chromatographic separation and electrochemical detector quantification method of Anderson, Young, and Cohen [J. Chromatog. 228: 155 (1982)]. Only pineal gland TRP was detectable in fetal rats under either vehicle or isoproterenol conditions. In contrast, by the time of birth 5HT concentrations were detectable in the pineal gland incubated with the vehicle. More importantly, incubation with isoproterenol caused reductions in pineal gland 5HT, increases in pineal gland NAS, and elevated concentrations of MEL in the culture media. These drug-induced changes in pineal gland MEL synthesis are similar to those observed in adult animals, and are presumably caused by isoproterenol-induced enhancement of N-acetyltransferase activity. Our data indicate that the immature pineal glands of newborn rodents are nevertheless capable of synthesizing and secreting MEL under appropriate conditions. (Supported by NIMH grant MH-31134.)

302.4 PHARMACO-ONTOGENY OF REWARD: ENHANCEMENT OF INTRACRANIAL ELECTRICAL SELF-STIMULATION BY d-AMPHETAMINE IN THE INFANT RAT. G.A. BARR* AND T. LITHGOW* (Sponsor: George Gourevitch). Dept. Psychiatry, Albert Einstein College of Medicine and Biopsychology Program, Hunter College, CUNY, New York, N.Y. 10021.

The rat is born both behaviorally and neurologically immature, but nonetheless is capable of learning. In the nest the pup must learn to approach, attach and suck from the dam's nipple. Furthermore, recent laboratory studies have shown that the infant rat is capable of responding operantly for milk infused into its mouth, or for direct electrical stimulation of the medial forebrain bundle, and classically to odors paired with milk infustion or other "activating" stimuli. Yet little is known of the physiological and neurochemical bases of reinforcement in the neonate. The present study found that d-amphetamine enhanced responding for intracranial electrical stimulation in a self-stimulation paradigm.

Three-day old Long Evans hooded pups were implanted with bipolar electrodes aimed at a number of forebrain sites. Eighteen hours later they were placed in a small test chamber and allowed to respond for stimulation (2.0 V, 150 Hz biphasic square wave of 250 µsec duration). Responses for each pup were counted on manipulanda that produced (S^+) or did not produce (S^-) the electrical stimulus. After 5 hours pups were injected with either 1.0 or 5.0 mg/kg of d-amphetamine HCl or the vehicle and tested for an additional 5 hours. Increased responding on both poles indicated enhanced activation while preferentially increased responding on the S^+ indicated increased reinforcement directed behavior.

The higher dose of amphetamine selectively enhanced responding for stimulation while the lower dose increased responding equally on both the S^+ and S^-. Histological analysis showed that responding on the S^+ increased most when the electrode was located in the anterior olfactory nucleus, nucleus accumbens, anterior medial forebrain bundle (MFB) or the globus pallidus. More posterior sites, including the MFB, were not enhanced by amphetamine.

Given the relative maturity of the mesolimbic dopamine system in neonatal rat pups, and that amphetamine enhanced responding for electrical stimulation, it can be postulated that reinforcement in the infant rat might be mediated, in part, by catecholamergic mechanisms. This suggestion is consistent with catecholamine theories of reward in adult animals. (Supported by PSC-CUNY Grant RF-6-63213 to GAB).

302.5 ONTOGENY OF REGIONAL BRAIN CARNITINE LEVELS IN RATS: R. G. Fariello, G. T. Golden, A. L. Shug*, Research and Neurology, VA Medical Center, Coatesville, PA; Thomas Jefferson Medical College, Philadelphia, PA and VA Medical Center, Madison, WI.

Carnitine (trimethylbetaine of γ-amino-β-hydroxybutyric acid) is a transmitochondrial carrier of free fatty acid chains in many human tissues and is structurally similar to choline and γ-aminobutyric acid (GABA). Carnitine and its acetylated derivative acetylcarnitine are unevenly distributed in mammalian brain. Recent evidence suggests that carnitine and its derivatives exert an action in the CNS either at the endoneuronal level, by entering into neurochemical patterns, or at the synaptic level, by interfering with transmission, or both. In particular interference with cholinergic and GABAergic functions has been suggested as the basis of several studies.

The present study was undertaken to examine the ontogenetic development of carnitine in discrete brain regions from birth to 100 days of age. Sprague-Dawley, Albino rats were decapitated at 15,20,30,40,50 and 100 days of age. The brain was quickly removed and dissected on ice into cerebellum (CB), brain stem (BS), thalamus (TH), caudate-putamen (CP), occipital-parietal cortex (O-P), midbrain (MB), basal forebrain (NBM) and remaining forebrain (FB) regions. Brain tissue was immediately frozen in dry ice and stored at -70°C until analyzed for total carnitine by a radiometric assay.

Total carnitine levels peaked at 20 days of age for all regions studied, excluding cerebellum, which showed a decrease. All brain regions examined showed a progressive decrease in total carnitine level from 20 days to 100 days of age.

At 15 days postnatally, the NBM and the CB have the highest content of total carnitine and the FB and O-P the lowest, with MB, TH, CP and BS intermediate. At 20 days of age, the rank order from highest to lowest was NBM>>TH>CP>O-P>BS, MB>CB, FB, at 30 days of age BS, MB, NBM>TH>CP, CB> O-P>FB. By 100 days of age regional analysis showed that O-P>BS>MB,CB>TH>CP>FB>NBM. The decaying rate of carnitine concentration was different in various regions with the highest rate for NBM (98%↓) and lowest for Brain Stem (62%↓).

Results will be discussed with reference to the ontogenesis of cholinergic neurons and cholinergic enzymes.

302.6 RAT BRAIN "MUSCLE-TYPE" ISOENZYMES OF CREATINE KINASE. O.C. Ramírez and G. Licea* Dept. Biochemistry, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, 07000 México, D.F.

Owing to its dimeric structure, cytosolic creatine kinase (CK) exists as three main isoenzymes: MM, MB and BB. Amino-acid composition, fingerprint analysis and immunochemical reactions show a high degree of structural homology between the isoenzymes of different species. The MM isoenzyme has been claimed to be an organ-specific, rather than a species-specific antigen. In differentiating skeletal muscle *in vivo* and *in vitro*, there is a considerable accumulation of CK activity. The transition of the CK isoenzyme pattern initiates with the BB-CK type, followed by the MB-CK and concludes with the mature MM-CK type in the adult of the species studied. It is thought that in vertebrate brain BB-CK is the sole form from the earliest stages throughout maturity (Eppenberger et al., Develop. Biol. 10:1, 1964). Evidence is presented here that as in skeletal and heart muscles, the rat brain synthesized catalytic forms of MM-CK and MB-CK in addition to the BB-CK brain type in quantities that permit the isoenzymes to be visualized by the $NADP^+$ coupled reactions technique. Total rat brain cytosolic (post 27,000 x g) CK specific activities were independent of the protein determination procedure and averaged 0.522±0.051 μmol creatine disappeared/min/mg protein. Heavy stained MM and MB-CK types were consistently found in the post 125,000 x g cytosolic brain fraction of rats whose weight ranged from 200 to 280 g. In no case spurious staining appeared in the blanks. Faint traces of the MM dimer have been found in occasions in adult dog, human, rabbit and guinea pig brain sources, but this is the first time that neural MB-CK isoenzyme has been found in a higher vertebrate.

Electrophoretogram of brain cytosolic CK isoenzymes

	Rat body range	Distance from the origin in cm±SD		
N	(g)	MM	MB	BB
18	200-280	(12)2.86±0.017	(7)3.03±0.35	(11)6.35±0.022

No. in parentheses indicate No. of rats showing a particular CK type. Some rats had more than one isoenzyme band.

302.7 CHANGES IN ACETYLCHOLINESTERASE ACTIVITY AND MOLECULAR FORMS IN A CLONAL NEUROBLASTOMA-GLIOMA HYBRID NG108-15 CELL LINE DURING DIFFERENTIATION. R. Ray, R.A. Kelly*, C.E. Murray*, and C.R. Storm*. US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

Treatment of NG108-15 cells with 1mM dibutyryl cAMP (Bt_2cAMP) for several days in culture produces marked alterations in various morphological and biochemical parameters characteristic of neuronal differentiation. Separate cultures of these cells, grown in the absence or presence of Bt_2cAMP, were analyzed for total amount of protein, and specific activity as well as molecular forms of acetylcholinesterase (AChE) at different days after initiation of cultures. Cholinesterase activity of these cells, assayed by a radiometric method using acetyl-1-^{14}C-choline iodide as substrate, was inhibited 90% by a specific AChE inhibitor compound BW284C51 ($IC_{50}=3x10^{-8}$M), indicating that the enzyme activity was predominantly AChE. After 6 days, Bt_2cAMP treated cultures contained one-third the amount of protein of untreated cultures; but, the specific AChE activity (nmoles ACh hydrolyzed/min/mg protein) of the treated cultures was three times (55 nmoles/min/mg) that of untreated cultures (20 nmoles/min/mg). AChE molecular forms in the cell extract prepared by sonication in a solution containing high salt concentration, ionic and nonionic detergents, and EDTA were characterized by their sedimentation coefficients in sucrose density gradients. Two molecular species with sedimentation coefficients of approximately 5S and 10S were found in all cultures, untreated or treated, after 3 or 6 days. After 3 days, when the untreated cultures were in the logarithmic growth phase and the treated cultures were incompletely differentiated, the relative contents of the 5S and the 10S molecular forms were approximately 80% and 20% respectively. However, after 6 days, when the untreated cultures were confluent and the treated cultures were highly differentiated, the 5S form decreased to 70% and the 10S form proportionately increased to 30% in both types of cultures. Work done in other laboratories have shown that AChE in brain also consists of these two molecular species, and their relative proportion alters similarly during development. These results show that activity and molecular forms of AChE are regulated in NG108-15 cells, and these regulatory mechanisms may possibly be involved in neuronal development and differentiation.

302.8 LAMINAR REVERSALS OF RECEPTOR BINDING PATTERNS IN CAT VISUAL CORTEX DURING THE CRITICAL PERIOD. C. Shaw, M.C. Needler* and M. Cynader. Depts. of Psychology and Physiology, Dalhousie University, Halifax, Nova Scotia.

We have recently demonstrated age-dependent changes in receptor properties such as number (B_{max}) and affinity (K_d) for various receptor populations in cat visual cortex during postnatal development. GABA, benzodiazepine, β-adrenergic, and acetylcholine (muscarinic) receptors all show increases in B_{max} during postnatal development with peak binding occurring during the physiologically-defined critical period. Changes in K_d were also observed for GABA, benzodiazepine, and acetylcholine receptors during the same period. We now report age-dependent reversals of the laminar patterns of binding for certain receptor populations during the critical period.

In vitro autoradiographic techniques were employed to examine the distributions of the following binding sites: [^{3}H] muscimol (GABA), [^{3}H] FNZ (benzodiazepine), [^{3}H] QNB and [^{3}H] NMS (muscarinic acetylcholine), [^{3}H] DHA (β-adrenergic), [^{3}H] glutamate (glutamate/aspartate/kainate), [^{3}H] AMPA (glutamate), and [^{3}H] pentagastrin/CCK-5 (cholecystokinin). Sections cut from the visual cortices of cats of various ages were incubated appropriately with the various ligands and apposed to LKB Ultrofilm.

Each of the ligands generated specific laminar binding patterns. Of these, three binding sites ([^{3}H] muscimol, [^{3}H] FNZ and [^{3}H] AMPA) showed invariant laminar binding patterns at each age examined. The other binding sites, however, showed age-dependent changes. For each of these cases binding was highest in cortical layer IV in young kittens (3 days-30 days postnatal), but reversed during the critical period so that by 95 days the binding sites were dominantly in the superficial and/or deep layers. During the same period the number of binding sites in layer IV decreased dramatically. Of the eight populations of binding sites examined, seven were initially densest in layer IV, the single exception being [^{3}H] AMPA which favored layers I, II and VI at all ages. Only [^{3}H] muscimol and [^{3}H] FNZ binding sites showed increases in number in layer IV during postnatal development.

Several possible mechanisms can be evoked to account for the observed reversals in binding pattern. Neuronal or glial elements with which the receptors are associated might migrate during postnatal development. Alternatively, subpopulations of each receptor might develop at different rates in the various laminae while being eliminated in layer IV.

302.9 DEVELOPMENT OF CHOLINERGIC AND PEPTIDERGIC PHENOTYPIC CHARACTERS IN THE RAT NEOSTRIATUM (NS) IN VIVO AND IN VITRO. H.J. Martínez,* C.F. Dreyfus and I.B. Black (Spon: K.A. Markey). Cornell Univ. Med. Coll., N.Y., NY 10021.

The neostriatum is a remarkable complex structure, consisting of biochemically distinct neuronal populations that have been implicated in a variety of clinical syndromes. To begin characterizing growth requirements of different striatal populations, we measured choline acetyltransferase (CAT) activity to monitor ontogeny of the cholinergic interneurons, and substance P to follow development of the peptidergic striatonigral neurons. There was notable synchrony in the development of these markers in the rat NS in vivo. CAT activity was detectable at birth, increased 6-fold after the first week to attain plateau levels by one month of age. Similarly, SP increased progressively after birth, describing a 5- to 6-fold increase by one month. To begin defining developmental regulatory factors, the NS from E20 (gestational day) fetuses was grown in organotypic culture. The NS was dissected free of surrounding brain tissue, sectioned into several pieces and placed in Maximov slide assemblies. During the 2 week culture period, CAT activity increased nearly 3-fold, suggesting that the cholinergic interneurons grew and developed in culture. In contrast, SP decreased markedly after 3 days in culture, reaching almost undetectable levels after 2 weeks. These observations suggest that the SP-containing, striatonigral projection neurons fail to develop normally, while the intrinsic interneurons do mature in culture. We are presently determining whether the peptidergic projection neurons require normal extrinsic targets for maturation in vitro.

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302.10 ONTOGENY OF PRO-ACTH/ENDORPHIN-DERIVED PEPTIDES IN THE RAT PITUITARY. S. M. Sato Dept. of Neuroscience, The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205

The content and molecular forms of immunoreactive pro-ACTH/endorphin-derived peptides from anterior and neurointermediate pituitary extracts of rats (ages; postnatal day 1 to 21) were determined using antisera directed against β-endorphin(10-19) and 16K fragment. Data are pmol/pituitary lobe (mean ± S.D.; N = 6, [N = 2, adults]).

	Anterior Lobe		Intermediate Lobe	
Age	16K frag.	β-endo.	16K frag.	β-endo.
Day 1	8 ± 1	6 ± 1	5 ± 0.1	7 ± 2
Day 3	7 ± 1	6 ± 3	8 ± 1	13 ± 2
Day 7	15 ± 2	10 ± 2	21 ± 2	32 ± 5
Day 14	21 ± 3	17 ± 5	36 ± 10	33 ± 8
Day 21	26 ± 4	18 ± 3	60 ± 9	76 ± 25
Adult(~300g)	130	79	1100	980

Gel filtration analyses (Sephadex G-75) demonstrated that the intermediate pituitary displayed a post-translational processing pattern throughout the postnatal period (day 1 to day 21) similar to that in the adult; peptides the size of αMSH (rather than intact ACTH) and β-endorphin (rather than intact β-LPH) were predominant peptides. In the developing anterior lobe, pro-ACTH/endorphin accounted for 40 to 50% of the total immunoreactive peptide, and material the size of αMSH was observed throughout the 3-week postnatal period. In contrast to the developing anterior pituitary, the adult anterior pituitary has only 10% pro-ACTH/endorphin and no detectable αMSH-sized material. RP-HPLC analyses of the molecular forms of αMSH-sized material in the intermediate lobe during postnatal development demonstrated that ~65% exists as diacetyl-ACTH(1-13)NH_2, and the rest as mono- and desacetyl-ACTH(1-13)NH_2; this distribution pattern is similar to the adult. By contrast, ~65% of the total immunoreactive αMSH-sized material in the developing anterior lobe is desacetyl ACTH(1-13)NH_2. The presence of αMSH and ACTH immunoreactive cells in both lobes of the adult pituitary and in the developing anterior pituitary has been examined using antisera directed toward the C-terminal α-amide of αMSH and the C-terminal of ACTH. As expected, all the cells in the intermediate lobe stained for both αMSH and ACTH and a population of ACTH-producing cells was detected in the adult anterior pituitary; no cells staining for αMSH were observed in the adult anterior pituitary. In contrast, the developing anterior pituitary displayed not only ACTH immunoreactive cells but also αMSH immunoreactive cells. Support: DA-00266, McKnight Fdn.

302.11 OPIATE RECEPTOR DISTRIBUTION IN THE BRAIN OF A NEWBORN RHESUS MONKEY. J. Bachevalier, J. B. O'Neill*, L. G. Ungerleider, and D. P. Friedman. Lab. of Neuropsychology, NIMH, Bethesda, MD. 20205.

The distribution of opiate receptor binding sites in the brain of a newborn rhesus monkey was mapped by in vitro autoradiographic localization of [^{3}H] naloxone. At one day of age, the infant monkey was anesthetized by intrahepatic injection of Na pentothal, and the brain was removed and frozen at -60°C. Cryostat-sectioned, unfixed, thaw-mounted tissue was first preincubated in 50 mM Tris, 150 mM NaCl, and 1 mg/ml BSA at 0°C for 30 min and then incubated in 2.6 nM [^{3}H] naloxone, 0.05M Tris-HCl, and 150 mM NaCl (pH 7.0) at 0°C for 1 hr. Nonspecific binding was determined in the presence of 1 μM levalorphan. The sections were fixed in paraformaldehyde vapors after incubation, apposed to LKB film, and exposed for 13 weeks at 20°C. The autoradiographs at selected levels through the newborn brain were compared to those from two adult monkey brains that had been processed in the same way.

A comparison between the newborn and adult brains revealed that the adult distribution of opiate receptor binding was already present at birth in allocortical areas and the hippocampal formation. These primitive areas showed conspicuously higher levels of opiate binding than the neocortex in both the newborn and adult brains. In the neocortex, by contrast, differences between the infant and adult brains were seen. Whereas the adult brain was characterized by areal-specific laminar patterns in primary sensory, motor and premotor, and dorsolateral prefrontal areas, the newborn brain was not. In the newborn brain, only the primary visual cortex had a distinct laminar pattern of labeling; in all other neocortical areas the infragranular layers showed relatively high levels of opiate receptor binding. In the adult brain, polysensory areas (e.g. PG and TF) showed greater labeling density than modality-specific sensory areas, but this density difference was not apparent in the newborn brain. At the subcortical level, the density and pattern of opiate receptors in the infant and adult brains were similar, with a patchy mosaic of labeling in the striatum, high levels of labeling in certain amygdaloid and thalamic nuclei, and an absence of labeling in the mamillary bodies.

The findings suggest that the distribution of opiate receptors in the macaque brain is adult-like at birth in limbic structures but is not yet fully developed in neocortical areas.

302.12 GLYCOCONJUGATE METABOLISM DURING NEUROGENESIS AND SYNAPSE FORMATION. J.R. Moskal*, A.E. Schaffner and G. Rougon*, (SPON: M. Zatz) LCB, NIMH, LNP, NINCDS and Lab. Immunol. INSERM-CNRS, Case 906, Marseilles, France.

The oligosaccharide moieties of cell surface glycoconjugates may play a role in cell migration, directed axonal growth and appropriate synapse formation. Experiments were undertaken to identify specific glycosyltransferases (GT) that may regulate these processes by the use of the mouse neuroblastoma x rat glioma hybrid cell line, NG108-15. Three specific GT activities were assayed: 1) a glycoprotein sialyltransferase using asialofetuin as acceptor (GPST); 2) the galactosyltransferase involved in the biosynthesis of the core blood group glycosphingolipid, lactoneotetraosylceramide (GALT); and 3) the sialyltransferase involved in the biosynthesis of the ganglioside GM3 (SAT-1). In one experiment the effect of retinoic acid (RA) (1.6 uM; 48 hr) or dibutyrl cyclic AMP (dBcAMP) (1 mM; 48 hr) on NG108-15 cells was examined. RA treatment elevated both sialyltransferase activities compared to controls: GPST, 4-fold; SAT-1, 10-fold. GALT activity was not affected by RA treatment but was elevated 40-50% by dBcAMP treatment. The high molecular weight form of the cell surface glycoprotein N-CAM was detected in both control and dBcAMP treated cells. However, cells treated with RA were found to have switched to the biosynthesis of a lower molecular weight form. Characterization of the enzyme(s) responsible for this change, probably caused by alterations in sialic acid content of N-CAM, are in progress. In another experiment, dBcAMP differentiated NG108-15 cells were cocultured with primary rat muscle cultures under conditions that give maximal synapse formation (Fishman, M.C. and Nelson, P.G., J. Neurosci (1981) 1, 1043). GALT activity was found to be elevated in these cocultures 3-fold over identically treated cultures of NG108-15 cells alone. Negligible activity was detected in similarly treated muscle cultures (minus NG108-15 cells). When dBcAMP-treated NG108-15 cells were cultured in the presence of muscle exudate GALT activity was also markedly elevated (2-fold) These results demonstrate that factors arising from the muscle cultures are able to significantly increase GALT activity. Experiments are in progress to further characterize these factors and attempt to establish a functional link between enzyme activity and synapse formation.

302.13 ACTION OF ECLOSION HORMONE ON THE NERVOUS SYSTEM OF THE MOTH MANDUCA SEXTA AT PUPAL ECDYSIS: INVOLVEMENT OF CYCLIC GMP. D.B. Morton* and J.W. Truman. (SPON: J.C. Weeks). Department of Zoology, University of Washington, Seattle, WA 98195.

At the end of each stage of their life cycle, insects go through a stereotyped behavioral sequence which allows them to shed the cuticle of the previous developmental stage. In various species of moths this behavior has been shown to be triggered by the peptide, eclosion hormone (EH), which acts directly on the nervous system. Previous studies on the silkmoth Hyalophora cecropia indicated that the actions of EH on the CNS are mediated by cGMP.

In the present study the mode of action of EH on the CNS of Manduca sexta at the time of pupal ecdysis has been investigated. During natural ecdysis a peak in the cGMP content of the nervous system is seen. This begins to rise soon after the release of EH, peaks at the beginning of ecdysis behavior and returns to the prestimulation level 90 minutes after ecdysis. A rise in the CNS levels of cGMP can be induced prematurely by injections of purified EH into prepupae. A significant increase in the levels of cGMP is seen 5 minutes after injection and the high level is then maintained until ecdysis. Injections of cGMP into prepupae will mimic the effect of EH in causing premature ecdysis in a dose-dependant manner.

The CNS is only behaviorally responsive to EH at certain times that just preceed the normal time of ecdysis. This sensitivity is then lost soon after ecdysis. Preliminary results suggest that EH does not produce a rise in cGMP in the CNS at all times during development. By comparing the ability of EH, given at various times, to stimulate ecdysis and to cause increases in cGMP it should be possible to gain insight into which steps; from the binding of EH by its receptors to the initiation of ecdysis behavior; are involved in the regulation of this sensitivity.

BIOCHEMICAL AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT II

303.1 EXPRESSION OF A BRAIN SPECIFIC mRNA AND PROTEIN DURING RAT BRAIN DEVELOPMENT. Dominique Lenoir*, Elena Battenberg, Floyd E. Bloom, J.Gregor Sutcliffe* and Robert J. Milner.* Research Institute of Scripps Clinic, La Jolla CA 92037.

We have used recombinant DNA techniques to select and characterize cDNA clones corresponding to brain specific mRNAs. The nucleotide sequences of several such clones have been determined, providing the amino acid sequences of the corresponding proteins. To detect these proteins, antisera were generated against synthetic peptides mimicking regions of the protein sequence. One such protein (1B236) defines a widely distibuted neuronal system and may be the precursor for a novel family of neuropeptides (Cell 33: 671, 1983). To determine the time course of expression of this and other brain specific genes during brain development, we have used protein and mRNA extracts and immunocytochemistry to study the brains of rats from the 14th embryonic day to 30 days postnatal, and from adult. 1B236 mRNA was detected by Northern blotting and quantitated by "slot blotting" using the p1B236 cDNA clone; 1B236 proteins were detected by Western blotting and quantitated by radioimmunoassay using appropriate antibodies against synthetic peptides. In whole brain extracts 1B236 protein and mRNA are first detected between 5 and 10 days after birth. At this stage the 1B236 protein is found only in extracts of hind brain and 1B236 immunoreactivity can be detected immunocytochemically only in cells and fibers in this region. With increasing age 1B236 immunoreactivity is found in progressively more rostral brain regions and the whole brain amounts of both 1B236 mRNA and protein increase to maximum values at 25 days postnatally. The whole brain content of 1B236 mRNA and protein at 25 days is approximately twice that of the adult and the immunocytochemical staining pattern for 1B236 resembles that of the adult. There is no detectable lag between the time of appearance of 1B236 mRNA and the expression of the corresponding protein, indicating that the developmental expression of the 1B236 gene is probably regulated at the level of mRNA transcription. Furthermore, the time course of 1B236 gene expression correlates with the expression of ID (identifier) gene elements, which we believe may regulate brain specific gene expression. The postnatal appearance of the 1B236 protein suggests that it is involved in functions that are specific to the adult brain. Supported by NIH grant #NS 20728 and grants from McNeil Pharmaceuticals and the Swiss National Foundation.

303.2 EFFECTS OF CHRONIC HALOPERIDOL ADMINISTRATION DURING DEVELOPMENT ON DOPAMINE AUTORECEPTORS IN YOUNG AND OLDER ADULT RATS. F. M. Scalzo & L. P. Spear. Department of Psychology and Center for Neurobehavioral Sciences, State University of New York at Binghamton, Binghamton, New York 13901.

Chronic haloperidol administration during prenatal and postnatal development has been shown to have pronounced behavioral and psychopharmacological effects. Spear et al. (1980) observed that chronically treated rats were hyperactive and displayed a decreased sensitivity to amphetamine as well as an increased sensitivity to haloperidol when compared with control animals. Shalaby and Spear (1980) observed that young adult animals chronically treated with haloperidol until weaning exhibited an attenuated low dose suppressant effect of apomorphine on locomotor activity. Given that the hypoactivity induced by low doses of apomorphine is presumably a result of autoreceptor stimulation, this work suggests that in the treated animals dopaminergic autoreceptor functioning may be disrupted by early haloperidol treatment. Alterations in dopaminergic functioning mediated by presynaptic mechanisms have also been reported to occur in aged animals and may be affected by chronic haloperidol treatment.

In the present study, gamma-butyrolacone (GBL)was used to investigate the effects of chronic haloperidol treatment on later dopamine autoreceptor functioning in striatum (ST), olfactory tubercles (OT), and nucleus accumbens (NAC) in young and older adult rats. Maternal injections of haloperidol or saline were given to Sprague-Dawley albino rats subcutaneously twice a day beginning on day 1 of gestation and continuing until weaning of the offspring on postnatal day 21. At 50-60 or 350-370 days postnatally, offspring were given injections of 1 mg/kg apomorphine or the ascorbate vehicle followed by 750 mg/kg GBL or the saline vehicle 40 and 35 minutes, respectively, before sacrifice. ST, OT, and NAC were assayed for DA, DOPAC, and HVA.

The results suggest that chronic developmental administration of haloperidol attenuates dopamine autoreceptor function, effects that are evident in young adulthood and are more pronounced later in life. In chronically treated animals sacrificed in young adulthood, apomorphine failed to attenuate GBL-induced increases in DA in ST and OT, whereas GBL treatment failed to induce increases in DA in chronically treated animals sacrificed later in life.

303.3 EVIDENCE FOR CALCIUM MEDIATED ACTION POTENTIALS IN PERIPHERAL NERVE OF RAT EMBRYOS. L. Ziskind-Conhaim, H.L. Fields. Dept. of Physiol. Neuro., Univ. Calif. Med. Sch., San Francisco, CA 94143.

The bundle of axons that innervates rat intercostal muscles is first present at 13 days of gestation (birth is at 21-22 days of gestation), one day after motorneurons are generated in the thoracic spinal cord (Nornes, H.O., Das, G.D. Brain Res. 73: 121, 1974). At that time nerve stimulation fails to evoke an endplate potential and muscle contraction. Nerve-muscle contacts become functional at Days 14-15. To determine whether at Day 13 the axons can generate action potentials, the proximal end of intercostal nerve was stimulated via a suction electrode and the evoked potentials were recorded extracellularly using 0.5-2 M heat-polished electrodes. At Day 13 and subsequent embryonic stages, suprathreshold stimuli consistently evoked a biphasic compound action potential (CAP). To determine the ionic mechanism of the CAP we studied the effects of different ions and channel blockers on the shape of the CAP. Bath-applied tetrodotoxin (0.1-1μM) or substituting sodium ions with choline or barium reversibly eliminated both phases of the CAP. Potassium channel blockers (tetraethylammonium (2-5mM) or 4-aminopyridine (1-2mM)) decreased the amplitude of the negative phase and often prolonged it .

To determine the contribution of calcium to the CAP cobalt (4mM) or cadmium (0.2mM) were added to zero-calcium recording solution, or 10mM cobalt was added to normal solution (containing 4mM calcium). Under these conditions the onset of the CAP was delayed and its duration prolonged. Both positive and negative phases had reduced amplitude. These effects were observed without blocking potassium conductance. We conclude that axons in embryonic peripheral nerve are capable of generating action potentials prior to establishing functional nerve-muscle contact. At this time the compound action potential is primarily sodium dependent with a small calcium component. Calcium contribution to the CAP can be due to a small fraction of calcium channels in axons that have a majority of voltage dependent sodium channel or a separate population of axons in which the action potential is primarily calcium dependent. Calcium component in the CAP disappears around birth.

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303.4 SYNAPSIN I IN PC12 CELLS: CHARACTERIZATION, AND EFFECTS OF NERVE GROWTH FACTOR. C.Romano, R.A.Nichols, and P.Greengard. The Rockefeller University, New York, NY 10021.

Synapsin I is a phosphoprotein present in most, if not all, neurons of the central and peripheral nervous system, where it is found in association with synaptic vesicles. It is not present in normal adult rat adrenal chromaffin cells. We have examined PC12 cells, a clonal line derived from a tumor believed to have originated from rat adrenal chromaffin cells, for synapsin I. These cells contain a molecule which is similar to synapsin I by several criteria: 1)it is specifically immunoprecipitated by several serum and monoclonal antibodies raised against synapsin I; 2) it is a phosphoprotein; 3) it is acid soluble; 4) partial proteolysis products of 35S-methionine or 32P-phosphate labeled synapsin I are identical to the products of correspondingly labeled rat brain synapsin I. Rat brain synapsin I is a discrete doublet of two related polypeptides, synapsin Ia and synapsin Ib; PC12 synapsin I is predominantly a singlet with a mobility between those of brain synapsin Ia and synapsin Ib.

Nerve growth factor (NGF) causes PC12 cells to acquire certain characteristics similar to those of sympathetic neurons. In the presence of NGF, synthesis of synapsin I is increased: this effect is detectable after several days and reaches a maximum of 3-5 fold over basal levels after three weeks. This induced material has a doublet character.

NGF also has a rapid, transient effect on synapsin I in PC12 cells. Within minutes of addition of NGF, synapsin I is no longer predominantly a singlet on SDS-PAGE, displaying instead increased heterogeneity and a lower average electrophoretic mobility. After several hours in the continued presence of NGF, the mobility returns to the basal pattern. Addition of dibutyryl cAMP or forskolin to PC12 cells also results in a rapid, transient effect, but in this case the mobility of synapsin I is increased. Pulse-chase experiments reveal that both of these effects are post-translational. The rapid, transient, and post-translational nature of these distinct effects suggests reversible, covalent modifications of synapsin I.

303.5 IDENTIFICATION OF AXONAL PROTEINS WITH POSSIBLE SIGNIFICANCE IN THE DEVELOPMENT OF THE NERVOUS SYSTEM. P. Sonderegger+*, P.F. Lemkin$, L.E. Lipkin$, P.G. Nelson+*. Lab. of Developmental Neurobiology, NICHD+ and Image Processing Section, NCI$, NIH, Bethesda, Md 20205, USA

The axonal functions that act in the formation of the neuronal network are phenomenologically described as elongation, sprouting, pathfinding, fasciculation and synapse formation. These processes have widely been shown to occur in close interdependence with the tissue that surrounds the growing axons. Hence, as a first step in a project aiming to determine the molecules involved in the implementation of axonal functions, we have identified axonal proteins whose synthesis is subject to environmentally induced changes.

Dissociated dorsal root ganglia (DRG) cells were plated in the center compartment of a compartmental cell culture system (Campenot, PNAS 74, 4516, 1977). The outgrowing axons crossed the barrier between center and side compartments through a thin film of medium, whereas the cell somas were retained in the center compartment. Peripheral or central glial cells and a mixed population of neuronal and glial cells from spinal cord were co-cultured with the DRG axons in the side compartment. The proteins synthesized under these different environmental conditions were metabolically labeled with [^{35}S]methionine added to the neuronal cell somas in the center compartment. After 40 h, the cellular material of the side compartments was harvested. The proteins synthesized in the neuronal somas of the center compartment and incorporated into the axons that extended into the side compartments were thus the only radioactively labeled proteins to be found in the side compartments. Two-dimensional SDS-polyacrylamide gel electrophoresis and computerized quantitation of the individual axonal proteins revealed that the co-cultured cells modulate the synthesis of a few axonal proteins of DRG neurons differentially. These proteins subdivide into groups with a common distinct modulation profile under the influence of peripheral glia, central glia and spinal cord cells.

Plasticity in the expression of axonal proteins in response to the different topographical provenance of the surrounding cells may reflect environmental modulation of axonal functions during the development of the nervous system. The proteins exhibiting a common modulation profile might be involved in the same, yet to date unknown, axonal functions.

303.6 NEONATAL DOPAMINE DEPLETING LESIONS SPARE ELECTRICAL SELF-STIMULATION OF THE BRAIN IN ADULT RATS James R. Stellar, Meg Waraczynski* and John P. Bruno. Department of Psychology and Soc. Rel., Harvard University, Cambridge, MA 02138, and Department of Psychology, University of Pittsburg, Pittsburg, PA, 15260.

In adult rats, interference with dopaminergic transmission through 6-hydroxydopamine (6-OHDA) lesions or receptor blockade is known to have severe debilitating effects on motor function and a variety of motivated behaviors, including self-stimulation for electrical stimulation of the brain (ESB). However, 6-OHDA lesions made in neonatal rats do not induce severe motor impairments, and tested as adults, the motor function of these rats is subsensitive to neuroleptics (Bruno, et. al. Neurosc. Abst., 9:1100, 1983). In this study, we extended this phenomenon to ESB self-stimulation.

Neonatal rats were lesioned at three days after birth with injections of 150μg of 6-OHDA into the lateral cerebral ventricles, 30 minutes after pretreatment with desmethylimipramine (25 mg/kg s.c.). After 5-7 months, rats were implanted with lateral hypothalamic electrodes and tested for self-stimulation. A reward summation function (RSF) was generated (Stellar et. al. Physio. & Behav., 18: 433-42, 1983) by measuring the bar pressing rate on a variable interval schedule of reinforcement (2.5 seconds) over a number of different ESB pulse frequencies. Two statistics were taken from the RSF: the maximal response rate and the ESB frequency required to sustain half maximal responding (the locus of rise). These two statistics reflect motor capacity and ESB reward pulse effectiveness, respectively.

The lesioned rats self-stimulated vigorously. Compared to controls (N=4) that were closely matched in ESB current (t=.45, df=5, p=NS), the lesioned group (N=3) had an insignificantly different locus of rise (t=.48, df=5, p=NS), but a significantly lower maximal response rate (61.9 vs 33.3 presses/min.; t=3.04, df=5, $p<.03$). Pimozide, administered in doses ranging from 0.125 to 1.0 mg/kg severely impaired control (N=2) responding at 0.5 mg/kg, as seen previously (Stellar, et. al., 1983). All lesioned animals were not significantly affected by this dose, and one rat was unaffected by the highest dose. Biochemical analysis revealed a striatal depletion of dopamine exceeding 95%. These results agree with previous work on simple motor function (Bruno et. al., 1983), and extend it to operant responding for rewarding ESB.

303.7 Molecular Alteration of Muscarinic Acetylcholine Receptors During Synaptogenesis Thomas H. Large, James Rauh* and William L. Klein Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60201

The assembly, positioning and regulation of receptor systems is important in the development of synaptic transmission. In the chick retina, the muscarinic acetylcholine receptor (MAChR) system is functional prior to synapse formation yet undergoes further changes in agonist binding properties during the period when synaptic contacts are formed. We have begun to investigate the possibility that post-translational modification of the MAChR during synaptogenesis might serve as a regulatory signal for changes in receptor positioning and/or receptor interactions with other functionally-related membrane proteins.

Initial biochemical characterization of 3H-PrBCM labeled MAChRs from hatched chick retina have shown it to have a molecular weight of 73 kD on SDS gels. In addition, digitonin solubilized MAChRs bound to wheat gern agglutinin (WGA) columns, indicating the receptor is a glycoprotein and contains sialic acid and/or N-acetylglucosamine residues. Treatment of retina membranes with neuraminidase, which removes sialic acid, decreased MAChR binding to WGA columns 75% but had little effect on molecular weight. Analysis of the molecular properties of MAChRs from bovine cerebral cortex indicated considerable homology with receptors from avian retina. MAChRs from cortex bound to WGA but not Con A lectin columns and had a molecular weight of 73-78 kD. Treatment of MAChRs with endoglycosidases D and H, in an attempt to remove all N-linked carbohydrate residues, resulted in a 4 kD decrease in molecular weight.

To examine what molecular changes in the MAChR may take place during synaptogenesis, we analyzed the isoelectric point (pI) of receptors at different stages of development of the avian retina. Prior to synaptogenesis, on embryonic day 13, the pI of MAChRs was 4.25 whereas MAChRs from hatched chick retina were distributed between a predominant form of pI 4.45 and a minor form of pI 4.25. The molecular transition appears to accur during synaptogenesis as MAChRs from E17 retinas were evenly distributed between the two forms. Modification also occurs in vitro as nine-day dissociated retinas grown in monolayer culture for seven days contained both high and low pI forms. Treatment of hatched chick retina membranes with neuraminidase increased the pI of MAChRs approximately .3 pH units but did not eleiminate the difference between the two forms. We are currently investigating possible changes in MW or phosphorylation.

303.8 EFFECTS OF MORPHOLOGIC DIFFERENTIATION ON CALCIUM CHANNELS AND CHOLINERGIC CHARACTER OF TE671 MEDULLOBLASTOMA CELLS. H.N. Siegel and R.J. Lukas, Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

The TE671 human medulloblastoma cell line grows in vitro as pleiomorphic cells with an undifferentiated appearance consistent with a primitive neuroectodermal origin. The original observation of the coexistence in culture (10% fetal bovine serum [FBS] in Eagle's minimum essential medium) of at least 5 morphologically distinct forms (McAllister et al., 1977) has been confirmed in our laboratory under culture conditions of 10% horse and 5% FBS in Dulbecco's Modified Eagle's medium (DMEM). Media replacement with DMEM supplemented with insulin, putrescine, transferrin, progesterone, and selenium (Bottenstein and Sato, 1979) results in the transformation of most cells to a more neuronal-like phenotype as determined by neurite formation, extension and arborization and the appearance of presumptive growth cones. Cells maintain this morphology for about 10 days and will remain stable longer if fresh media is added. Substitution with DMEM and 0.1% FBS slows, but does not halt, mitosis, and addition of 1 mM dibutyryl cAMP (but not 8 Br cAMP or 2 mM sodium butyrate) induces a rapid yet partially reversible transformation to distinctly neuronal-like cells. Extensive outgrowths of neuritic processes form synaptic-like figures reminiscent of axo-axonic and axo-somatic neuronal contacts. TE671 cells possess functional nicotinic receptors and high affinity binding sites for ^{125}I-α-bungarotoxin (Bgt) as well as binding sites for other neuroactive substances. They do not have significant choline acetyltransferase activity (Syapin et al., 1982). We find that the dihydropyridine ^{3}H-nitrendipine (Nit), a putative antagonist of voltage-gated calcium currents, reversibly binds with high affinity to intact cells. Most of the bound Nit (at 1 nM) is displaceable within 10 min by 1 uM nifedipine, with diltiazem less effective. Mature TE671 cells display approximately 100 fmol of specific Nit binding per mg cell protein. Binding sites for ^{125}I-α-Bgt similarly are of high affinity and density. The TE671 cell line holds promise for examining regulatory mechanisms of morphologic differentiation of neuronal phenotype.

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303.9 ACTIVITY-DEPENDENT K^+ ACCUMULATION IN RAT OPTIC NERVE RENDERED AMYELINATED: A DEVELOPMENTAL STUDY. C.L. Yamate* and B.R. Ransom (SPON: L. Eng). Dept. of Neurol., Stanford Univ. Sch. of Med., Stanford, CA 94305.

The adult mammalian CNS exhibits a "ceiling level" of 8-12 mM for the maximum $[K^+]_o$ which accumulates with neural activity. The development of this adult "ceiling level" has previously been studied in a simple model of the CNS, the rat optic nerve (RON). Neonatal nerves show an unusually large ceiling $[K^+]_o$ of 17.2 mM. This level gradually falls over the next two weeks to the adult level of 9.8 mM (Connors et al., Science 216:1341, 1982). Glial cells appear in RON with a similar time course to the development of the adult ceiling level suggesting that glia may be involved in "setting" this level. To test this possibility, we attempted to reduce the number of glia in RONs by treatment with the mitotic inhibitor, 5-Azacytidine (5AZ), and then compared the developmental changes in ceiling level in treated and control RONs. Treated animals received intraperitoneal injections of 5AZ (3.3 mg/kg) on postnatal days 1, 3, 5 and 7. RONs of different ages were analyzed with regard to maximum evoked $[K^+]_o$ ("ceiling level") and peak $[K^+]_o$ elicited by single supramaximal stimuli as previously described (Connors et al., 1982). Experiments were done at 37° and bath $[K^+]$ was 3 mM.

The developmental time course for K^+ ceiling level was significantly altered in the 5AZ-treated animals. Mean ceiling levels in RONs 6-12 days old were 13.0±3.3 (treated) and 8.2±2.2 mM (controls), and in RONs 13-21 days old these levels were 10.7±0.5 (treated) and 7.1±0.6 mM (controls). Since the developmental time course for peak $[K^+]_o$ evoked by single supermaximal stimuli was not significantly different in treated and control animals, the delay in appearance of the normal adult ceiling level was not likely to depend upon changes in the rate of K^+ release (reflected by K^+ rises to single stimuli). Compound action potentials in treated RONs up to 13 days old had a triphasic appearance characteristically seen only in RONs < 5 days old, suggesting a delay in axonal maturation or myelination. Preliminary observations reveal that 5AZ may markedly reduce myelination in comparison to control RONs of the same age. A quantitative evaluation of glial elements in treated nerves is underway. We conclude that 5AZ treatment produces a striking maturational delay in the establishment of adult ceiling level as well as myelination. Supported by NIH grants NS 15589 and NS 00473 from the NINCDS.

303.10 DEVELOPMENT OF VASOPRESSIN BINDING SITES IN THE LONG-EVANS RAT BRAIN: AN AUTORADIOGRAPHIC STUDY F.M. Petracca*, D.G. Baskin, J. Diaz, and D.M. Dorsa (Spon: W. Catterall). Departments of Pharmacology, Psychology, Biological Structure, and Medicine, University of Washington, Seattle, WA 98195, and the Veteran's Administration, Seattle WA 98108.

Arginine^8-Vasopressin (AVP) is a peptide hormone involved in maintenance of fluid homeostasis and blood pressure. Anatomical and behavioral evidence support the hypothesis that AVP may also act as a neurotransmitter or neuromodulator. In addition, this laboratory and others have recently reported putative receptors for vasopressin, localized in discrete regions of the adult rat brain which are known to receive vasopressinergic innervation. These areas include the lateral septum, central nucleus of the amygdala, olfactory tubercle, ventral tegmentum, and nucleus of the solitary tract.

The vasopressinergic system has also been implicated in the development of the nervous system, since homozygous Brattleboro rats, which do not secrete this peptide, have diminished brain growth. The purpose of the present study was to examine the ontogeny of vasopressin receptor sites in the rat brain, as these receptors may have an important role in mediating trophic influences of vasopressin.

Receptor sites were localized using a method of binding ^{3}H-AVP to brain slices of Long-Evans rats from postnatal Day 0 to adult. 20-micron, slide-mounted, sections were incubated for 30 minutes in 200 microliters of Tris buffer solution containing 2 nM ^{3}H-AVP in the presence or absence of 2 uM unlabeled AVP. The slides were rinsed, dried, and placed in contact with LKB Ultrofilm for 30-45 days.

Results indicated a changing pattern in distribution of AVP receptors over development. Binding in Day 0 brains was minimal and limited to the dorsal septum and caudate nucleus. In contrast, binding in the adult forebrain was localized in the lateral septum and was not present in dorsal septum nor in caudate. Binding sites corresponding to those seen in the amygdala in adult brains were not seen until day 4 in the developing brain. By day 5, intense binding was apparent in the dorsal hippocampus, followed by a reduction in binding in succeeding days up to adulthood.

The anatomical distribution of vasopressin binding sites in the rat brain changes significantly throughout development, suggesting a trophic role for vasopressin in the ontogeny of specific brain structures.

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303.11 IN-VIVO VOLTAMMETRIC ANALYSIS OF THE DEVELOPMENT OF SPONTANEOUS AND AMPHETAMINE-INDUCED DOPAMINE (DA) RELEASE IN RATS.

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Using semi-differential in vivo voltammetry, the spontaneous release of DA was measured in the 21-22 day and 35-38 day rat pup neostriatum and compared with the spontaneous release found in the adult rat. The effects of d-amphetamine in these three age groups were also measured.

Male Sprague-Dawley rats were injected with chloral hydrate (300 mg/kg i.p.) and body temperature maintained with a heating pad. Electrode implantation followed standard stereotaxic techniques. Surface-modified graphite electrodes, selective for catecholamines, were inserted into the neostriatum. Semi-differential voltammetric measurements were performed by scanning from -0.1V to +0.5V vs. Ag/AgCl and measuring the peak current centered at +0.13V. Scans were repeated once every 10 min. At the end of each experiment, an in vitro standard calibration curve was run so that current values for release could be related to known concentrations of DA.

Spontaneous DA release in the 21-22 day group was approximately 20% of adult values while that in the 35-38 day group was approximately 43% of adult values.

After a stable baseline was obtained, rats were injected with amphetamine (1 mg/kg s.c.). In the adult rat, DA release reached a maximum (+85%) compared to baseline after 40 min. In the 35-38 day group a maximum increase (+30 to +65%) in DA release occurred after 40 min. However, in the 21-22 day group amphetamine produced an initial slight increase in DA release followed by a decrease in DA release of -30 to 40% over the three hour testing period.

These data demonstrate a developmental increase in spontaneous DA release when compared with adult levels. These data also suggest a difference in response to indirectly acting DA agonists in the developing rat pup at 21-22 days, when compared with that seen in the adult rat.

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303.12 IDENTIFIED NEURONS PRODUCE ACETYLCHOLINESTERASE BEFORE THEY SPROUT AXONS IN THE ZEBRAFISH. Eric Hanneman* and Monte Westerfield (SPON: P. O'Day). Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Can neurons acquire a transmitter phenotype before they grow axons? We examined this question for two classes of identified neurons in the zebrafish central nervous system, the Mauthner cell (M-cell) and the primary motoneurons (PMNs). Since both classes have been shown to possess cholinergic properties, we studied the onset and distribution of acetylcholinesterase (AChE) activity to determine when these neurons can first degrade acetylcholine (ACh).

Cholinesterase activity specific for ACh can first be detected by 15 hours post-fertilization. Stained neurons are bilaterally symmetric and segmentally arranged in the hindbrain and spinal cord.

By 18 hours post-fertilization, the number of AChE positive cells in each hemisegment has increased to 2-4 cells situated in a tight cluster. The hindbrain contains several of these segmentally arranged clusters. One is situated at the rostral aspect of the ear, and corresponds in position to the location of the M-cell. Neurons in clusters in the spinal cord correspond in number, size, and position to the early PMN somata. The temporal development of the spinal cord staining proceeds in a rostral-caudal direction.

Other workers using several approaches have confirmed that the peripheral processes of the PMNs first leave the spinal cord between 18 and 19 hours post-fertilization. The M-cell initiates axonal outgrowth at 20 hours. Based on these data, we conclude that the PMNs and probably the M-cell acquire AChE activity, and perhaps a cholinergic phenotype, prior to the time that they begin to grow axons and make synaptic connections. (Supported by NIH GM07257 and NSF BNS8103573.)

303.13 SHORT- AND LONG-TERM BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF NEONATAL SYSTEMIC 6-OHDA AND 6-OHDOPA TREATMENTS IN MICE. M. J. Forster and Z. M. Nagy, Dept. of Psychology, Bowling Green St. Univ., Bowling Green, OH 43403.

Studies were conducted to determine the development and persistence of behavioral and neurochemical effects of lesions to central and peripheral norepinephrine (NE) systems. Mice received systemic injections (60 µg/g) of the neurotoxins 6-hydroxydopamine (6-OHDA) or 6-hydroxydopa (6-OHDOPA) or vehicle on postnatal days 1, 3, and 5. Acquisition and 24-hr memory of a discriminated escape task were examined in these mice at 13, 19, 30, or 100 days of age. At each age, acquisitioin training consisted of 25 trials in which shock-offset occurred upon reaching the correct goal in a T-maze. A similar session was given 24-hr later to these mice, as well as to appropriate groups of yoked controls in order to assess memory of prior training. In a second behavioral test, additional mice selected from each of the 3 postnatal conditions were tested for locomotor activity after receiving 4 mg/kg of d-amphetamine at 13 or 100 days of age. Finally, NE in cortex/hippocampus and whole heart was determined fluorometrically in additional groups of treated and control mice aged 13, 30 or 100 days. The assays indicated that mice treated with 6-OHDA or 6-OHDOPA had comparable reductions of cortex/hippocampus NE content at all ages tested, with those receiving 6-OHDA also showing a reduction of whole heart NE. On the activity test following d-amphetamine, both groups treated with the neurotoxins exhibited smaller increases in activity compared to vehicle controls. On the learning and memory task 13-day-old treated mice displayed poorer acquisition and memory than controls, while neurotoxin-treated mice failed to show any learning or memory deficits at the older ages when compared to controls. The present findings suggest that early 6-OHDA and 6-OHDOPA treatments may delay maturation of those NE systems involved with learning and/or memory of tasks using an aversive stimulus. However, recovery of behavioral function was rapid after the early lesions, notwithstanding the long-lasting NE depletion and loss of pharmacological responsiveness. The behavioral recovery may indicate post-lesion reorganization of the damaged NE systems, or compensation by other transmitter systems. The findings may also reflect the fact that NE modulation of learning or memory processes becomes less critical with increasing age. (Supported by NICHHD grant HD-01945 to Z. M. N.)

304.1 IN VITRO PROLIFERATION AND DIFFERENTIATION OF NEURAL CREST CELLS IN A DEFINED CULTURE MEDIUM. M. Sieber-Blum and H.R. Chokshi*. Dept. of Cell Biology and Anatomy, The Johns Hopkins Univ. Sch. of Med., Baltimore MD 21205.

Cultured quail neural crest cells give rise to several differentiated phenotypes and thus represent an attractive experimental system for the study of cell differentiation. However, so far the analysis of the underlying regulatory mechanisms has been hampered by two factors, 1) the contamination of the cultures with cells of noncrest origin and 2) the complex growth medium that contained two ill-defined components, horse serum and chick embryo extract. The introduction of clonal cultures (Sieber-Blum, M. and Cohen, A.M., Develop. Biol. 80: 96, 1980) eliminated the first problem. We now report the formulation of a defined culture medium that supports proliferation and differentiation of quail neural crest cells in primary cultures. Neural tubes were explanted into collagen- and fibronectin-coated culture dishes and cultured in medium MCDB 202 supplemented with insulin, transferrin, cortisone, gonadal hormones, vitamins, and trophic factors. The explanted neural tubes adhered to the substratum and the neural crest cells emigrated in the usual fashion. Cell proliferation was slower than in medium containing serum and embryo extract. After 4-6 days all cultures contained pigment cells that were densely packed with melanin granules and had the typical appearance of melanocytes. Twenty-five percent of the cultures contained adrenergic neurons as indicated by intense formaldehyde/glyoxylic acid-induced catecholamine fluorescence. Neurons had characteristic long, varicose processes with growth cones and had rounded cell bodies that tended to aggregate with each other. We believe that the development of this defined medium will prove useful in the study of neural crest cell differentiation under controlled conditions. (Supported by USPHS Grant HD15311)

304.2 DIVIDING ROD PRECURSORS IN THE GOLDFISH RETINA: THREE DIMENSIONAL RECONSTRUCTION FROM SERIAL, ELECTRON MICROSCOPIC AUTORADIOGRAPHS. P.A. Raymond and P.K. Rivlin*, Univ. of Michigan, Department of Anatomy & Cell Biology, Ann Arbor, MI 48109.

When larval goldfish hatch, their retinas contain postmitotic, undifferentiated cones but no rods. Cones rapidly differentiate during the first few days of larval life. Meanwhile, rods begin to appear; they are produced by dividing, specialized progenitors - rod precursors - scattered across the retina. Rod precursors continue to divide throughout larval and into adult life, adding new rods to the growing retina. These dividing cells, intercalated among differentiated retinal neurons, are unique and have not been described previously. The purpose of this study was to identify rod precursors in the larval retina, to characterize them ultrastructurally and to reconstruct their morphology.

Dividing rod precursors were labeled by ^{3}H-thymidine injections in late larval/early juvenile goldfish (26-51 days posthatch). Serial thin sections were prepared from the center of the eye, and every 20-25th section in the series was processed for autoradiography. Labeled rod precursors were identified, located in the serial set, traced on transparencies, digitized and reconstructed using a locally developed computer graphics program.

Our results show that rod precursors are typically located on the vitread side of the layer of photoreceptor nuclei (outer nuclear layer, ONL). They extend a single, long apical process through the ONL toward the external limiting membrane, a feature reminiscent of the primitive neuroepithelial cells from which they were derived. Short, ridge-like processes extend laterally from the cell body. At 12 hr survival postinjection labeled nuclei are in interphase, by 24 hr many labeled nuclei are mitotic and by 48 hr there are many labeled pairs; this implies that the cell cycle of rod precursors is 24 to 48 hr long. Some cells that had just withdrawn from the mitotic cycle, as evidenced by their proximity to a cluster of labeled precursors and by the morphological features of their nuclei (intermediate between paler, larger, irregularly-shaped precursors and darker, smaller, more ovoid rods), contain perinuclear (immature) ribbons and accumulations of synaptic vesicles indicative of their differentiation into rods. The above results support the premise that rods in the fish retina are derived from a special class of neuroepithelial cell.

304.3 CELLULAR LINEAGES FORMING THE ZEBRAFISH NERVOUS SYSTEM. C.B. Kimmel, R. Warga*, and R.D. Law*. Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

We have studied neuronal lineages originating from zebrafish blastomeres injected with lineage tracer molecules. Identified blastomeres that arise by the same division pattern in different embryos make variable contributions to the CNS and other tissues, forming longitudinally oriented patchy stripes of labeled cell clusters. Within the CNS of many embryos, pairs of clusters are present on opposite sides of the midline, and sets of clusters are often distributed periodically, with segmental spacing, along the neuraxis. Adjacent clusters frequently contain neurons of the same or similar morphology. These features are independent of when the clone was founded (through the 2,000 cell stage) and they are independent of the region of the CNS that the clone occupies.

To learn how these clonal distributions arise, we have followed portions of sublineages of fluorescently labeled blastomere clones in live embryos by low light-intensity video microscopy. Cells of a blastomere clone begin to spread apart from one another during gastrulation and become dispersed into labeled single cells and small cell clusters that are scattered among unlabeled, unrelated cells. In contrast to the original blastomere clones, sublineages that arise at the gastrula stage frequently produce only a single tissue type. The segmentally distributed clusters observed at later times are subclones founded by closely related gastrula cells that migrate apart from one another. A bilateral pair of clusters observed in the CNS is derived from a single sublineage founded by one cell in the late gastrula.

We interpret these results to mean that at the blastula stage zebrafish cells have not been restricted to form a particular type of tissue or to migrate to a particular location in the embryo. Developmental restrictions could first arise in cells during gastrulation and be transmitted to clones of their descendants. (Supported by NSF grant no. BNS-8112477.)

304.4 DO GRANULE CELLS CONTROL THE EXPRESSION OF CEREBELLIN IN THE DIFFERENTIATING PURKINJE CELL? J.R.Slemmon*,J.L.Hempstead* and J.I.Morgan* (SPON:E.Cantor). Dept. of Phys. Chem. and Pharm.,Roche Inst. of Molec. Biology, Nutley,NJ 07110.

The cerebellum of rat and mouse contains two unique peptides, cerebellin and des-Ser^{1}-cerebellin. The peptides have been isolated, sequenced and subsequently synthesized by solid phase techniques. Sequence-directed antibodies have been raised to synthetic cerebellin conjugated to thyroglobulin. Antisera were then affinity purified against cerebellin coupled to activated Affi-Gel 10. Radioimmunoassay and chemical analysis show both cerebellin species to be enriched in the synaptosomal fraction of cerebellum. Immunocytochemical studies show cerebellin to be localized in the soma and dendrites of Purkinje cells. During development cerebellins first appear at 5 days after birth. Levels then rise rapidly to a maximum at day 25 post partum after which they decline to a stable adult level. This biochemical picture is mirrored by immunocytochemical analysis where immunoreactivity is first observed about 4 days after birth in the immature Purkinje cells. As the granule cell population migrates from the external granular layer to its adult stratum, cerebellin expression increases rapidly in the arborizing dendrites of the Purkinje cells. This situation is the same in the mouse. Chemical analysis of the cerebella of 3 granule cell mutations of mouse (Reeler, Weaver, Staggerer) showed almost no cerebellin. Purkinje cell mutants (pcd, Nervous) showed only a slight diminution in peptide levels, commensurate with their direct loss of Purkinje cells. This indicated that granule cells might control cerebellin expression. Immunocytochemical studies on Reeler showed only a few cerebellin-immunoreactive Purkinje cells that had apparently reached their correct anatomical locus. There were also two foci of weakly reactive immature cells in the Reeler cerebellum. In preliminary experiments, the Weaver mouse showed more immunoreactivity than Reeler. Since neither mouse had cerebellin peptide it is concluded that the antibody is binding to cerebellin precursor in these studies. Thus we suggest that the Reeler mouse does not express cerebellin precursor or peptide whereas the Weaver does express precursor but has aberrant processing machinery. Since the common denominator is a loss of granule cells or some intrinsic defect in these neurons,we feel that an understanding of the biochemical dysfunctions of cerebellin metabolism will provide keys to the nature of the control of Purkinje cell differentiation.

304.5 DEVELOPMENTAL STAGE SPECIFIC ANTIGENS IN THE CEREBRAL CORTEX OF RODENTS. M. Yamamoto, A. Boyer*, G. Schwarting*. Div. of Neuropathol. & Biochem., Shriver Ctr., Waltham, MA 02254

To study the molecular mechanisms underlying the development of the cerebral cortex, monoclonal antibodies were produced against embryonic brain. Homogenized tissue from E15-18 rat forebrain was used as immunogens. Among the antibodies produced, antibody (7A) reacts intensely with embryonic cortical anlagen in the rat & mouse. Its temporal & spatial distribution patterns were studied in the mouse using immunocytochemistry. From E11 to E13, the full thickness of the cortex is stained throughout its lateral extent, but the medial wall shows no staining until E17. On E15, shortly after cortical plate (CP) and the intermediate zone (IZ) appear, the CP does not stain and the deepest level of the IZ stains weakly. Intense immunoreactivity is confined to the ventricular (VZ) and subventricular zones (SVZ). At PO(E19), no staining is seen in the cortex. Other immunoreactive regions of the CNS during embryonic development are follows:a narrow ventro-lateral sector of the hypothalamus, the ventral wall of the inferior colliculus and the ventricular germinal zone in the medulla. Not all the germinal zones are labelled. The anlagen of the basal ganglia (BG) and the thalamus are negative until E17 but stain subsequently. No staining is seen in the spinal cord or the dorsal root ganglia. Immunoreactivity in rat brain is identical except that positive staining is observed in the BG at E15.
In primary tissue culture prepared from E15 rat forebrain, 80% of the cells are immunoreactive 2hr after plating. The percentage of positive cells decreased to 50% after 5 days. Immunoreactivity appears to be confined mainly to the plasma menbrane. Precursers for both neurons & glia appear to be positive. After 5 days in culture, immunoreactivity is observed in some vimentin-positive glial cells.
Immunoprecipitation does not show any reactive protein band in embryonic tissue. However, immunoblotting on HPTLC plate reveals three immunoreactive bands of neutral glycolipids. No reaction is seen with gangliosides.
Supported by NIH grant #HD04147-15.

304.6 NEURORETINAL PROLIFERATION AND DIFFERENTIATION FOLLOWING VIRUS INDUCED TRANSFORMATION IN VITRO. Mary F.D. Notter and Piero C. Balduzzi*, Departments of Anatomy and Microbiology, University of Rochester Medical Center, Rochester, New York, 14642.

Embryonic retina has been shown to contain a recognition factor which allows dissociated retinal cells to reassociate with appropriate cell types in vitro and in vivo. We have begun a study to examine this cellular recognition of embryonic retinal cells following transformation by RNA tumor viruses and to assess the effects of the virus on individual retinal cell types. Seven day embryonic chick neuroretina was trypsin dispersed, placed either in monolayer culture or rotation-mediated, reaggregation culture and infected with Rous sarcoma virus (RSV). Determination of viral functions and cell transformation indicated that both retinal cell monolayers and spheroids produced virus and that cells were mitotically stimulated and morphologically transformed. Retinal cell spheroids in culture for 24 hrs. could be transformed by virus after cell associations had been formed, while transformed monolayer cultures could form spheroids when cells were removed from their surface and placed in rotation. Specific staining for nerve specific enolase, toxin binding, vimentin, and acetylcholinesterase of monolayer cultures indicated the presence of neurons and muller cells two weeks following cellular transformation; while tyrosine hydroxylase and choline acetyltransferase activities were detected biochemically in both virus infected monolayers and spheroid cultures. However, histological examination of virus infected, retinal spheroids demonstrated that typical cell associations were not made. Mitotic figures were present and cells appeared as swirls around a central cell core. These preliminary data suggest that although cellular differentiation as determined by enzyme activity and specific staining can occur following viral transformation, normal neuroretinal architecture is not achieved.

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304.7 DEVELOPMENT OF THE CHOLINERGIC BASAL FOREBRAIN NUCLEI IN THE RAT EMBRYO. Alan Fine. Neurochemical Pharmacology Unit, Medical Research Council, Cambridge CB2 2QH UK.

The large cholinergic neurones of the basal forebrain nuclei (medial septal nucleus, MS; vertical and horizontal limbs of the diagonal band of Broca, DBv and DBh; and nucleus basalis magnocellularis, NBM) are the main source of cholinergic innervation to the telencephalon. To study the development of these nuclei in the embryo, and to examine their ontogenetic relations, rat embryos at various developmental stages were fixed by transcardiac perfusion with, or immersion in, 4% paraformaldehyde in phosphate buffer. Cryostat sections of embryos at each developmental stage were cut in three orthogonal planes, and stained for acetylcholinesterase (AChE) by a silver-intensified modification of the acetylthiocholine method of Koelle. AChE has been shown to be a reliable marker of cholinergic cells in the basal forebrain of the adult rat (Levey, A.I. et al., Neuroscience 9, 9, 1983). AChE+ NBM precursors are first visible beneath the striatal eminences of crown-rump length 12mm embryos (gestational day 14), far in advance of the AChE+ cells of the adjacent neostriatum. The cells appear to migrate both rostrally and caudally; by the 15mm stage they have extended to the region of the presumptive zona incerta and to the DBh. AChE+ precursors to MS appear by the 16mm stage as a distinct nucleus, without evident ontogenetic link to NBM or DBh. Shortly thereafter, DBv precursors can be seen adjacent to, and presumably derived from, MS. Other AChE+ nuclei of the diencephalon and mesencephalon arise independently of the basal forebrain nuclei.

304.8 A "clonal" analysis of somatosensory cortex barrel formation using chimeric mice. Daniel Goldowitz, Dept. of Anatomy, Thomas Jefferson University, Philadelphia, PA 19107.

These studies were designed to address the question: Do developmental mechanisms exist in the mammalian cerebral cortex by which clonally related neuroblasts become translated from the ventricular zone to compose cortical structural units? The possibility of a clonal development for discrete assemblies of mammalian somatosensory neurons was examined in the posteromedial barrel subfield (PMBSF) of chimeric mice. Chimeric mice are made by combining 4-8 cell embryos from two sets of parents that vary in a determinant that allows us to identify a given cell as belonging to one parental genotype or the other. β-glucuronidase levels, detected by histochemical means, was used as the cell marker to determine the genotype-of-origin of each neuron. B6-beige (bg/bg) (high β-glucuronidase activity), C3H (low β-glucuronidase activity), and B6 bg/bg $\longleftrightarrow$ C3H chimeric mice were perfused with 4°C, 4% paraformaldehyde. A tangential cortical slice including PMBSF region was sectioned with a vibratome, stained for β-glucuronidase activity, embedded in epon, sectioned at 3-5 μm, counterstained with methyl green and analyzed. Based upon β-glucuronidase histochemistry of control brains, cortical barrel cells could be assigned to the high or low genotype with a confidence of about 98%. Several individual barrels of somatosensory cortex were examined in six chimeras comprised of varying proportions of B6 bg/bg and C3H cells. Four chimeras, two of each whose barrel neurons were predominantly derived from one genotype or the other, were carefully analyzed to optimize the detection of coherent clones and possible enzyme transfer. The analysis of these four chimeras and control mice led to the determination that the β-glucuronidase marking system was applicable to PMBSF neurons. Neurons from the two genotypes were found to be distributed in very similar proportions amongst all the barrels examined from a given chimera. Furthermore, a serial-section analysis of single barrels provided no evidence for any obvious non-random distributions of neurons from one genotype in a single barrel. Such evidence does not support the notion of a unique founder population of neurons for individual barrels (or groups of barrels), and supports a notion of extensive cell mixing and epi-genetic events in the determination of individual barrels in the mouse somatosensory cortex.

304.9 REGULATION OF CEREBELLAR PURKINJE CELL NUMBER: A QUANTITATIVE ANALYSIS OF LURCHER CHIMERIC MICE. K. Herrup and T.J. Diglio*. Department of Human Genetics, Yale Medical School, New Haven, CT 06510.

Studies of the means by which the developing nervous system regulates cell number usually focus on the phenomenon of naturally occurring cell death. Equally as important, though less often studied, are those factors that regulate how many cells are generated to begin with. Data obtained by quantitative analysis of the Purkinje cell (PC) population of chimeric mice suggest that the number of cells produced is an intrinsic property of the progenitor cells selected as PC ancestors. The C3H/HeJ inbred mouse strain has 81,600 (± 3%) PCs in each cerebellar half. In C57BL-Lc ↔ C3H/HeJ chimeras all C57BL/6 (B6) PCs are destroyed during postnatal development by the cell-autonomous action of the lurcher (Lc) gene. The remaining PCs are thus all C3H/HeJ but the counts of these cells in six half cerebella do not assume all possible values. Rather they increase in "quanta" of 10,200 (±3%) PCs. These quanta are the numerical evidence of developmental clones of PCs each of which descends from a single progenitor cell selected early in development. The C3H/HeJ mouse itself contains 8 clones of 10,200 PCs per cerebellar half. In this study, an identical series of chimeras were produced using B6 instead of C3H/HeJ as the wild-type strain. This inbred mouse strain contains 92,000 (±3%) PCs per cerebellar half. In the C57BL-Lc ↔ B6 chimeras, numerical quanta were seen as before. Instead of each quantum (or clone) containing 10,200 PCs, however, these animals showed evidence of a clone size of 9,200. The B6 mouse itself thus contains 10 clones of 9,200 PCs each. For both C3H/HeJ and B6, the size of a clone did not vary in lurcher ↔ wild-type chimeras that ranged from mostly lurcher to mostly wild -type. The ratio of lurcher to wild-type cells, therefore, is not a factor in this analysis. Our conclusions are, first, that the number of cells of a given neuronal type can be regulated by a change in either the number of cells in any one clone (i.e. clone size) or the total number of clones in the population (or both). Second, the data suggest that the initial size of a clone is an intrinsic property of the progenitor cell that gives rise to it. This implies that, in addition to an early autonomy of morphological fate (i.e. cell type), the cells of the early CNS may be committed to a numerical fate as well. Supported by the March of Dimes (1-763) and the NIH (NS 18381).

304.10 DISTRIBUTION OF IMMUNOHISTOCHEMICALLY IDENTIFIED AVIAN SENSORY NEURON SUBPOPULATIONS IS CORRELATED WITH AXIAL LEVEL. M. F. Marusich*, K. Pourmehr*, and J. A. Weston* (SPON: G. Ciment). Dept. of Biology, University of Oregon, Eugene, OR 97403.

We have developed a monoclonal antibody (designated (SN1) that binds to a subpopulation of quail sensory neurons. Detectable levels of the antibody do not bind to other PNS neurons (sympathetic or enteric), nor to any CNS neurons tested (spinal cord, cerebellum, cerebrum, retina). The antibody binds to live cells, which indicates that the identified antigen is a cell surface molecule. The epitope is resistant to trypsin, pronase, and sialidase digestion. The time course of appearance of $SN1^+$ sensory neurons was determined with dorsal root ganglia (DRG) pooled from all axial levels, dissociated and cultured for 16-20 hours. $SN1^+$ neurons are first detectable in cultures of ganglia from 7-day embryos, and the proportion of positive cells remains low (less than 10%) through day 11. Between embryonic days 11 and 12, the proportion of $SN1^+$ neurons in cultured ganglia increases rapidly to about 50%, which is maintained at least until hatching on day 16.

Immunohistochemical examination of tissue sections of newly-hatched quail revealed that sensory neurons within brachial and lumbar DRGs, which innervate limbs, are predominantly $SN1^-$, whereas sensory neurons within thoracic DRGs are predominantly $SN1^+$. We are using this marker to test the hypothesis that segmental sensory innervation patterns are specified prior to the extension of neurites into the periphery.

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LIMBIC SYSTEM

305.1 DEVELOPMENT OF GABAergic NEURONS IN THE RAT HIPPOCAMPAL FORMATION. L. Seress*, C.E. Ribak, G.M. Peterson and W.H. Oertel. Dept. of Anatomy, Univ. of Calif., Irvine, CA 92717 and Dept. of Neurology, Technical Univ., Munich, FRG.

A number of studies have demonstrated the presence of GABAergic neurons in the adult rat hippocampal formation. Briefly, GABAergic neurons are found in all cell layers, and include the large basket cells in strata granulosum and pyramidale that give rise to a pericellular axonal plexus around the granule and pyramidal cells. The development of the GABAergic neurons was studied in three types of preparations: ^{3}H-thymidine autoradiographic, Golgi and immunocytochemical. The first group of rats received single injections of ^{3}H-thymidine at different postnatal ages from 6 hours to 12 days, were sacrificed at 50 days of age and standard autoradiograms of their brains were prepared. Neither basket cells nor typical GABAergic hilar neurons were labeled. However, small size neurons were occasionally labeled in the dentate gyrus molecular layer but it was difficult to determine if they were displaced granule cells or local circuit neurons. These data suggest that most GABAergic neuronal types are probably formed prenatally. In Golgi preparations basket cells and other local circuit neurons are first observed at 3-5 days postnatal (dpn). These neurons are similar to the ones found in adult brains in regard to somal size and position but they appear to have shorter and thicker dendrites. Since various types of GABAergic cells are found at this early age, immunocytochemical preparations for glutamate decarboxylase (GAD), the synthesizing enzyme for GABA, were analyzed at various postnatal ages to determine when these neurons have the functional capacity to mediate GABAergic inhibition. At 10 dpn, no GAD+ reaction product was observed in the hippocampus although the basal ganglia displayed axonal labeling in these same preparations. By 14 dpn, GAD+ cells and puncta were present in both the dentate gyrus and Ammon's horn. Some GAD+ cells (2-3 per section) displayed a Golgi-like staining of their dendrites at this age. Between 14 and 16 dpn, the number of GAD+ cells had doubled to reach the adult levels. In the older animals studied in this series (18-24 dpn), the number of GAD+ cells did not change significantly but the intensity of axonal and dendritic staining was increased. Based on these observations, the GABAergic neurons of the hippocampal formation probably begin to function around 14 dpn which is much later than the time when they can be identified structurally in Golgi preparations. However, these immunocytochemical data are consistent with the development of inhibition in the hippocampal formation as determined with electrophysiological methods. (Supported by NIH grant NS-20228 and the Klingenstein Foundation.)

305.2 SEPTAL HYPERREACTIVITY: A MULTIVARIATE ANALYSIS OF ITS RECOVERY AND THE ROLE OF THE SUPERIOR CERVICAL GANGLION. R. G. Thompson and F. H. Gage. Psychology Dept., Texas Christian University, Fort Worth, TX 76129.

Gross lesions of the septal area are well known to elicit a dramatic increase in the animal's response to tactile stimuli. The neural basis of this hyperreactivity remains a subject of controversy since remission of the syndrome has been shown to occur spontaneously and can be greatly facilitated by experiential and pharmacological manipulations. Coincident with damage involving the medial septum is the documented ingrowth of sympathetic fibers from the superior cervical ganglion (SCG) into the hippocampus and other select CNS structures. Several studies have recently reported a functional role of the SCG ingrowth in post-lesion behavior patterns. The objectives of the present study were: 1) to discriminate between the behavior of septal-lesioned rats at various times after surgery, and 2) to discriminate between the behavior of septal-lesioned rats with an intact SCG and septal-lesioned rats with bilateral ganglionectomies.

Animals received sham or bilateral ganglionectomies two weeks prior to septal surgery. After receiving bilateral lesions of the septum, the animals were randomly assigned to groups representing 3, 7, 15 and 30 days of recovery. The animals were tested on their reactivity to a light air-puff, bodypoke and handling using a traditional rating scale. The animals' response to inescapable footshock was also obtained yielding the animals' response latency, duration and response integral.

The results indicate that the behavior of septal-lesioned rats is virtually indiscriminateable from controls 30 days after the lesion. There also did not appear to be any major basis for discriminating between the ganglionectomized and non-ganglionectomized septals at 3, 7 and 15 days post-lesion However, these two groups could be discriminated at 30 days. This discrimination was largely due to the effect of the ganglionectomy in blocking the continued recovery of the animals' magnitude of response to the footshock. This may be interpreted to suggest that the anomalous ingrowth of sympathetic fibers into the central nervous system after septal lesions is counterproductive to the recovery of some behavioral functions while not affecting the remission of other closely related behaviors.

305.3 METABOLIC CORRELATES OF KINDLING-INDUCED CHANGES IN THE REWARDING EFFICACY OF HIPPOCAMPAL STIMULATION: A 2-DEOXYGLUCOSE AUTORADIOGRAPHIC STUDY. K. A. Campbell. Dept. of Psych., Univ. of Pennsylvania, Phila., Pa., 19104.

Previous studies have demonstrated that the usually very slow acquisition of hippocampal (HPC) self-stimulation (8-14 daily 30-min sessions) can be greatly faciltated (to 1-3 sessions) by a prior program of HPC kindling (Brain Research, 159, 458, 1978). It has been hypothesized that the reinforcing consequences of HPC stimulation may develop as the stimulation-induced activity propagates more widely through pathways potentiated by prior kindling. The metabolic tracer [^{14}C]-2-deoxyglucose (2DG) was used to measure the functional activity resulting from electrical stimulation of the HPC, comparing uptake in a group of confirmed HPC self-stimulators with a group of stimulation-naive rats. A group of implanted, unstimulated naive controls were also used.

17 rats were implanted in the dorsolateral HPC (CA-3), of which 6 were trained to self-stimulate: optimal parameters using 0.5 sec trains of 0.1 msec square, monophasic-negative pulses were found to be 80 μA at 75/sec. During 2DG uptake (30 μCi i.p.), the confirmed self-stimulators were either allowed to self-stimulate (n=2) or given programmed stimulation (5 sec ISI; n=4) at the above optimal parameters for self-stimulation. Stimulation-naive rats were either given the same programmed HPC stimulation (n=7), or were not stimulated (n=5). After 45 min, the animal was immediately sacrificed, perfused, and the brain frozen, sliced, and prepared for autoradiography; details of preparation and computer densitometry of radiographs have been described in Gallistel et al. (Neurosci. Biobehav. Rev., 1982).

Radiographic analysis indicated that CA-3 stimulation in a confirmed self-stimulator results in widespread increases in metabolic activation throughout HPC fields CA-1,2,3 and posterior subiculum bilaterally, whether stimulation was self-administered or programmed; the dentate gyrus was unaffected. In naive rats, the programmed CA-3 stimulation produced very limited activation, in most cases restricted to the region around the electrode tip, with metabolism in contralateral or distal HPC not markedly different from unstimulated controls.

The present results demonstrate that HPC stimulation in experienced HPC self-stimulators (kindled animals) produces considerably more widespread activation of HPC than does the same HPC stimulation in naive rats which would not be expected to exhibit self-stimulation.

305.4 NONLINEAR CHARACTERISTICS OF HIPPOCAMPAL PERFORANT PATH-DENTATE SYNAPTIC TRANSMISSION ARE DIFFERENT FOR SYNAPTIC AND ACTION POTENTIAL CURRENTS. R.J. Sclabassi, J.L. Eriksson*, and T.W. Berger, Depts. of Psychology, Psychiatry and Neurosurgery, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Random impulse trains of electrical stimulation delivered to the perforant path (PP) are highly effective in revealing nonlinear characteristics of synaptic transmission to the dentate gyrus (DG) as measured by amplitude of the granule cell field potential population spike (Berger et al., Soc. Neurosci. Abstr., 1983). The population spike is known to reflect currents generated by granule cell action potentials. We have now analyzed the effect of random impulse trains of electrical stimulation on the population epsp component of the field potential recorded in the dentate dendritic layer, which is known to reflect synaptic currents. Results show that epsp and spike field potentials respond significantly differently to variation in frequency of perforant path input.

A computer-generated random interval train (Poisson distribution, λ=2 Hz, band-width=0.2-1000 Hz) was used to determine intervals between 4064 successive stimulations (0.1 msec duration) of the PP in the anesthetized rabbit. Field potentials generated in the dendritic layer of DG were analyzed for amplitude of the epsp. Our previous analysis of population spike amplitude had revealed that, with respect to second-order nonlinearities (i.e., the effect of just the preceding interval), the population spike exhibits: i) total inhibition at high frequencies (100-200 Hz), ii) facilitation of a maximum of 100-200% at 10-15 Hz, iii) partial inhibition (10-30%) at 1.5-3 Hz, and iv) no significant alterations at frequencies less than 1 Hz. In contrast, the population epsp shows: i) little or no inhibition at the same high frequencies of stimulation, ii) much less facilitation (30-40%) that is maximal at higher frequencies (30-35 Hz) than for the population spike, and iii) no inhibition at 1.5-3 Hz, so that the epsp responds linearly to all frequencies less than 40-50 Hz. Even greater differences between epsp and spike potentials are revealed when third-order nonlinearities are examined (i.e., the effect of the two preceding intervals). While amplitude of the population spike can vary by as much as 50-60% depending on the absolute value of the two preceding intervals, the population epsp can be predicted almost entirely on the basis of the preceding interval alone. Thus, the epsp exhibits virtually no third-order nonlinearities. Supported by The Whitaker Foundation and MH 30915.

305.5 LONG-TERM POTENTIATION ALTERS NONLINEAR CHARACTERISTICS OF HIPPOCAMPAL PERFORANT PATH-DENTATE SYNAPTIC TRANSMISSION. T.W. Berger, J.R. Balzer*, J.L. Eriksson* and R.J. Sclabassi. Depts. of Psychology, Psychiatry and Neurosurgery, Univ. of Pittsburgh, Pittsburgh, PA 15260.

High-frequency stimulation of perforant path (PP) afferents to the hippocampal dentate gyrus (DG) is known to be associated with long-term potentiation (LTP) of PP-DG synaptic efficacy. In addition to this effect on the amplification or "gain" of the synapse, we investigated the possibility that LTP also alters the frequency responsiveness of PP-DG synapses, and thus, their nonlinear properties.

A computer-generated random interval train (Poisson distribution, λ=2 Hz, band-width=0.2-1000 Hz) was used to determine intervals between approximately 1000 successive single-shock (0.1 msec duration) stimulations of the PP in the anesthetized rabbit. Field potentials generated in the cell body layer of DG in response to each pulse in the train were analyzed for amplitude of the population spike. The train was delivered both before and 30 min after LTP was induced by high frequency stimulation (400 Hz) of the PP.

Prior to induction of LTP, Wiener kernel analysis revealed second-order nonlinearities described previously (Berger et al., Soc. Neurosci. Abstr., 1983). That is, at high frequencies of stimulation (100-200 Hz), action potential generation is completely inhibited such that no spike components of the field potential are recorded. At moderate frequencies in the range of 10-15 Hz, a facilitation of 100-200% in spike amplitude is seen, while at lower frequencies (1.5-3 Hz), a significant depression of 10-30% occurs. No nonlinearities in synaptic transmission occur with frequencies less that 1 Hz. LTP results in a marked alteration of these second-order nonlinear properties. The most significant post-LTP change is a reduction in the facilitation normally seen at moderate frequencies of stimulation. The average maximum facilitation seen after LTP was 42% vs. 158% pre-LTP. In addition, the range of stimulation intervals at which facilitation is observed is also altered (40-322 msec pre-LTP; 38-254 msec post-LTP). Finally, inhibition of the population spike in response to high frequencies of stimulation is reduced following LTP. Post-LTP inhibition of the population spike to stimulation frequencies in the range of 25-60 Hz is at least 15% less than the inhibition seen before LTP. These data show that changes in the potency of a synaptic connection also can result in significant alteration of its nonlinear response properties. Supported by The Whitaker Foundation and MH 30915.

305.6 EFFECTS OF EXPERIENCE DURING SUCKLING ON WEIGHT AND VOLUME OF RAT HIPPOCAMPUS. Catherine P. Cramer and John C. Hueston*. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Post-weaning environmental complexity can have effects on behavior and brain anatomy, physiology, and chemistry. Recently we have shown that acquisiton of maze tasks is profoundly affected by experiences during suckling; rats allowed to shift from nipple to nipple learned the task in half the number of trials required by rats not allowed to nipple-shift (Cramer, 1982). Because preweaning nipple availability has such profound effects on behaviors with known neural correlates, in these experiments we examined brain development following differential rearing.

Rats were reared from postnatal Days 5-25 in litters of 5 by dams with either 12 or 4 nipples (to encourage or discourage nipple-shifting, respectively). Weights of whole brain, cortex, cerebellum, and hippocampus were determined on Day 25. While total brain weight, cerebellar weight, and cortical weight were the same for both groups, the hippocampal weight of rats reared with only 12 nipples was significantly greater than that of rats reared with 4 nipples.

A histological analysis was conducted to determine if there was an accompanying change in the volume of the hippocampus following differential suckling experience. Rats were reared as described above. Hippocampal volumes were estimated from tracings of Kluver-Barrera-stained sections. Hippocampi of rats reared with 12 nipples were significantly larger than those of rats reared with 4 nipples.

(Supported by PHS grant MH38436.)

305.7 ALTERATIONS IN PAIRED PULSE INHIBITION AND POTENTIATION IN CA1 OF THE RAT HIPPOCAMPUS AS A FUNCTION OF VARIATION IN CONDITION AND TEST PULSE INTENSITY. C.J. Rogers, J.F. Ott, L. Jakeman, D.W. Walker, and B.E. Hunter. Department of Neuroscience, University of Florida, Coll. of Med. and V.A. Medical Center, Gainesville, FL 32610.

This study was conducted in an effort to dissociate the effects of feed-forward inhibition (FFI) from those of recurrent inhibition (RI). Paired-pulse stimulation was delivered to CA1 of the hippocampus in either orthodromic/orthodromic (O/O) or antidromic/orthodromic (A/O) pairs. In addition to the use of two stimulus configurations, we sought to further dissociate FFI from RI by comparing the pattern of population spike (PS) responses by varying the conditioning and test pulse current intensities.

Experiments were conducted in Long-Evans hooded male rats. Electrodes were placed in stratum radiatum at the CA2-CA1 border for orthodromic stimulation and in the alveus for antidromic stimulation. Glass micropipettes were positioned in stratum oriens to record PS responses. Orthodromic condition pulse intensities of 100%, 75%, 50%, and 25% of the PS maximum were used as well as the current intensity at PS threshold and 50% of the EPSP amplitude at PS threshold. Antidromic current intensities used were 100%, 75%, 50%, 25% and 0% of the maximum antidromic response obtained. Test pulse current intensities were 50% and 25% of PS maximum.

A/O and O/O PP stimulation produced PS amplitudes comparable to those previously reported. The major results of our studies were: 1) As the intensity of A/O condition pulses increased, the magnitude of inhibition increased. 2) As the intensity of O/O condition pulses increased, both magnitude and duration of inhibition increased, though an asymptote was reached which was well below the highest current levels. 3) Subthreshold O/O condition pulses produced little inhibition but resulted in potentiation equal in magnitude to that found with greater current intensities though shifted to shorter inter-pulse intervals. 4) O/O stimulation produced an inhibition of test PS responses greater in magnitude than that produced by A/O stimulation. The results of our experiments suggest that the combined effects of FFI and RI are not simply additive. Rather they appear to interact in a synergistic manner.

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305.8 USING PARALLEL WIRES TO RECONSTRUCT THE DISTRIBUTION OF ELECTRICAL POTENTIAL ACROSS THE HIPPOCAMPAL SLICE. T.J. Teyler, L.J. Cauller* and B.L. Wilhite.* Neurobiology Dept., N.E. Ohio Univ. College of Medicine, Rootstown, Ohio 44272.

Lamellar slices were prepared from rat hippocampus in the usual way (Teyler, Brain Res. Bull., 1980, 5, 391). Special electrode arrays were fabricated out of uninsulated, thin (25-50μ), stainless steel wire. WIREs were strung in parallel (50-100μ separation) across a glass ring which was used in place of a pool in the slice chamber. The slice, mounted on filter paper, was suspended over the electrode array by floating it on the surface of the pool in such a way that the slice was directly, although delicately, contacting the array of WIREs.

This type of electrode was conceived by analogy with the CAT scan technique of reconstructing two-dimensional distributions from one-dimensional ray integrals. For this reason, we call this type of electrode a Wire Integrating Ray Electrode (WIRE) array. The signal recorded by each WIRE is hypothesized to be the integral of all potentials encountered along its exposed path.

We recorded radiatum-evoked potentials with the WIRE array while it was projected across the slice in one of two directions, parallel or perpendicular to the CA1 cell body layer. The characteristic CA1 depth profile was observed when the WIREs were parallel to the cell body layer. Potentials recorded by WIREs projecting along the stratum radiatum were reversed with respect to the oriens projection. Furthermore, in slices where the WIRE array was perpendicular to the cell body layer, we recorded null potentials. In all cases, slice vitality was confirmed with conventional micropipette recordings.

We suggest that the integrated potential from the perpendicular projection is nulled because the reversed portions of the depth profile cancel. Also, current source-density analysis of the depth profile is justified by our findings because no lateral profile was found. We plan to derive the profile for additional projection angles and to apply the algebraic reconstruction technique to these recordings.

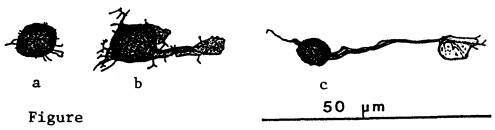
305.9 DEVELOPMENT OF GRANULE CELLS IN THE RAT OLFACTORY BULB. K. Kishi*, H. Ojima*, A. Masuda* (SPON: Y. Shinoda) Dept. of Anatomy, Tokyo Medical and Dental Univ., School of Medicine, Tokyo, Japan 113.

Several H^3-thymidine autoradiographic studies (Altman '69 Hinds '68) have shown that most of granule cells postnatally originate from the proliferative subventricular zone (SVZ) of the mammalian forebrain, and migrate into granular layer (GrL) of the olfactory bulb. But, the process of this cyto-differentiation has not been elucidated. To clarify this Wistar rats at 1,3,5,10,15,21,37, and 60 postnatal days were stained with Nissl and Golgi methods.

In Nissl-stained, sagittal sections of the rat forebrain, SVZ surrounding the olfactory ventricle extends from the anterior horn of the lateral ventricle to the center of the olfactory bulb where SVZ is surrounded by the GrL.

In Golgi sections, SVZ-cells are classified into three groups. The first is round cells with many fine microvilli (Fig. a), and two daughter cells. They are possibly mitotic cells. The second is somewhat large cells, approximately 10 μm in diameter, with many cytoplasmic processes. Some of this group have a thick process with growth cone (Fig. b). They are possibly cells of glial cell-line. The third is bipolar cells, approximately 8 μm in diameter, with smooth contour. They have a long apical process under 50 μm in length and a short basal process. Most of the apical processes with large growth cones orient towards the olfactory bulb (Fig. c). They are migrating from the anterior horn of the lateral ventricle to the GrL. They occupied over 50 % of Golgi-stained SVZ-cells in the rats from 1 to 21 postnatal days, and 17 % at 37 and 60 days. They are possibly progenitors of granule cells. After their migration into the GrL, they differentiate to the difinitive granule cells ; their somata enlarge; apical processes elongate, branch, sprout many gemmules, and become the peripheral processes; basal processes become basal dendrites.

References: Altman, J. 1969 J. Comp. Neur. 137: 433-458. Hinds, J. W. 1968 J. Comp. Neur. 134: 287-304.



Figure

305.10 THE DENDRITIC MORPHOLOGY OF HIPPOCAMPAL CA3 PYRAMIDAL NEURONS: CELL TYPES AND ENVIRONMENTAL INFLUENCES. J. M. Juraska, J. Fitch* and L. Washington*. Dept. of Psychology, Indiana Univ., Bloomington, IN 47405.

We have previously demonstrated that the sexes differ in dendritic response to rearing in complex (EC) and isolated (IC) environments. In the visual cortex male rats show greater dendritic differences following differential rearing than female rats in some cell populations (Juraska, Brain Res. 295:27, 1984), while females exhibit greater plasticity than males in the granule cells of the hippocampal dentate gyrus (Juraska, et al., Neurosci. Abst. 13:947, 1983). In the present experiment we examined the dendritic tree of neurons from the hippocampal CA3 area, the area that receives input from the dentate granule cells via the mossy fibers, in male and female rats reared in EC or IC for one month postweaning. Neurons were sampled from coronal sections of Golgi-Cox stained tissue.

A concentric ring analysis revealed only a few, seemingly random, differences. However, we observed that there were two morphologically distinct cell populations in our pyramidal neuron sample. The most numerous type has a comparatively long apical shaft that ends in a sparse dendritic tree. The second cell type has a short, thick apical shaft and a dense dendritic tree. A third type with a dendritic tree similar to that of the short apical shaft pyramidals exists without any apical shaft; these cells were not sampled in the present study. When the concentric ring analysis included the type of pyramidal cell (long vs. short apical shaft) there were statistically significant differences between the cell types in both their apical and basilar fields. There also were significant interactions in apical and basilar trees between the cell types and environmental conditions, with long apical shaft cells unaffected by the environment and short apical shaft cells from IC rats having more dendritic material than those from EC rats. This interaction is preliminary since the short apical shaft neurons comprise less than 25% of the 390 neurons sampled. No sex differences were found. Another replication in a separate group of animals is currently being performed to examine a larger sample of the short apical shaft pyramidal neurons.

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305.11 POSTNATAL DEVELOPMENT OF A CELL SURFACE MARKER FOR LIMBIC SYSTEM NEURONS. H.L. Horton*, A. Turtz*, P. Levitt (SPONS: N. Berman). Dept. Anatomy, The Med. Coll. of Pennsylvania, Phila., Pa. 19129.

Specific chemical markers that are distributed on related cells may serve in part to link developing neurons into distinct functional systems. A monoclonal antibody (2G9) produced against adult hippocampal membranes specifically labels neurons of the limbic system, providing strong evidence for molecular specificity among functionally related neurons (Levitt, Science 223: 299, 1984). Using this monoclonal antibody, we have performed an immunocytochemical analysis in the developing rat to determine whether the limbic neuron marker is expressed in the same regionally specific fashion during the time of pathway and synapse formation in the limbic system.

Albino rats at eight different ages from birth to postnatal day 28 (P28) were prepared for immunofluorescent staining. 2G9 immunoreactivity is distributed in a specific fashion at all ages examined. Nuclei or cortical areas that are not immunoreactive in adults are not stained during postnatal development. However, fiber tracts, which are not stained in adults, are immunoreactive for a short time postnatally. At birth, cells in only a few regions are stained. Very light immunoreactivity is distributed along the surface of cells and diffusely in the neuropil in the septum, n. accumbens, amygdala, anterior thalamic n., and some regions of the hypothalamus and brainstem. Limbic cortical areas and the hippocampus are not immunopositive at birth. The most intense staining is seen in fiber tracts, particularly the corpus callosum, internal capsule, stria terminalis and fornix. By P2, sparse staining appears in the hippocampus and frontal cortex. By P7, most regions that are 2G9-positive in the adult contain some immunoreactive cells. By P12, the distribution of staining within cortical regions and nuclei is identical to the adult, although the intensity is not as great. In addition, most tracts are no longer immunoreactive. By P19, the pattern and intensity of staining are fully matured. The data indicate that the limbic system neuron marker develops in a specific fashion, restricted to those cells that will express the determinant in the mature CNS. The early, transient presence of immunoreactivity in fiber tracts is suggestive of the antigen participating in the formation of limbic system connections.

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DEVELOPMENT AND PLASTICITY: TROPHIC AGENTS II

306.1 βNGF IN SYNAPTOSOMAL FRACTIONS OF MOUSE CEREBRAL CORTEX? J. Lakshmanan*, M.E. Weichsel, Jr., H. Kim*, R. Tarris* and D.A. Fisher*,Perinatal Research Laboratory, Harbor-UCLA Med. Ctr, Torrance, CA 90509

We examined βNGF immunoreactivity in synaptosomal fractions isolated from mouse cerebral cortex during various stages of development. Pooled cortex tissues were homogenized (1:9 w/v) in ice-cold 0.32M sucrose with a glass-teflon homogenizer (0.25mm clearance). The synaptosome enriched fraction was then prepared by differential centrifugation, followed by a discontinuous sucrose density gradient technique established for rodent brain. Synaptosomal pellets were homogenized in phosphate buffered saline (PBS), pH 7.4, and centrifuged to obtain the 115,000 xg supernatant referred to as "synaptosomal extract (SE)". βNGF concentration was quantified using a high affinity antiserum (kd = $\sim$1.7 x 10^{-11} mole/liter) generated against purified mouse submandibular gland βNGF with assay sensitivity of 16-20 pg/tube.

In 21 day old mice, the relative concentrations of βNGF in whole brain (B), cerebral cortex (C), and SE were (mean ± SEM); B=61±2, C=59±5, SE=279±13, pg/mg protein, respectively. Intraventricular injection of βNGF (5 ng/animal=6 x whole brain content), 16 hr prior to sacrifice did not significantly alter βNGF concentration in SE; control vs βNGF injected animals were 279±13 and 230±24 pg/mg protein, respectively.

βNGF concentrations in SE prepared from nerve terminals of cerebral cortex during various stages of development were as follows:

Postnatal Age (days)	12	16	21	26	32
pg βNGF/mg SE protein*	683 ±113	503 ±80	298 ±46	276 ±30	245 ±32
mg SE protein/gm cortex*	0.74	0.96	0.85	0.78	0.81
*Mean ± SEM	±0.05	±0.08	±0.04	±0.05	±0.01

When SE prepared from 12 day old mouse cerebral cortex in RPMI-1640 buffer was bioassayed in a PC-12 cell system (in the presence of 1% horse serum for 24 hr), a positive neurite outgrowth response was observed; the effect was blocked by βNGF antibody. Conclusion: 1) βNGF concentration is high in synaptosomal fractions compared to whole brain or cerebral cortex. 2) Intraventricular βNGF injection does not influence synaptosomal βNGF. 3) Endogenous cerebral synaptosomal βNGF concentration is highest in the nerve terminals of 12 day old mice. 4) βNGF concentration in the synaptosomal fraction decreases with increasing age.

306.2 EFFECTS OF GANGLIOSIDE TREATMENTS ON LESION-INDUCED SPROUTING BY THE SEPTODENTATE PATHWAY. B. Fass, J.J. Ramirez*, S.E. Karpiak and O. Steward. Neurosurgery Dept., Univ. Virginia Med. Sch., Charlottesville, VA 22908; Psychology Dept., College of St. Benedict, St. Joseph, MN (JJR); Division of Neuroscience, NY State Psychiatric Inst., N.Y., N.Y. (SEK).

Exogenous gangliosides (glycosphingolipids) have been shown to enhance regenerative growth in the peripheral nervous system (e.g., Gorio et al., 1983) and to promote behavioral recovery after some types of CNS injury (e.g., Karpiak, 1983). The present study examined whether gangliosides also enhance one type of lesion-induced growth in the CNS; namely, the sprouting by septodentate fibers induced by unilateral lesions of the entorhinal cortex. Such fibers are rich in acetylcholinesterase (AChE), and their sprouting response to entorhinal lesions can be revealed by a quantifiable histochemical stain.

The intensity of AChE staining in the denervated neuropil of the dentate gyrus was measured microspectrophotometrically at 3,5,7, or 10 days postlesion in treated (total brain gangliosides, FIDIA Research Laboratories; 50 mg/kg, im) and untreated adult rats (3-7 animals/group/survival interval). The dentate gyrus contralateral to the lesion was used as an internal control. Treatments began on the day before surgery and were given daily until sacrifice.

In the untreated group, there was an intensification of staining as early as 3 days postlesion and a substantial increase between 7 and 10 days. In the ganglioside-treated group, the general pattern of changes in staining was similar, except that the increases were consistently smaller at each survival interval. The groups differed significantly overall ($F=4.65$; $df=1,23$; $p<.05$); posthoc contrasts revealed an intergroup difference at 10 days postlesion ($p=.05$), but not at the earlier intervals.

The present findings suggest that although ganglioside treatments enhance behavioral recovery after entorhinal lesions (Karpiak, 1983), they do not enhance sprouting by one of the pathways which participate in the sprouting response. Since exogenous gangliosides have been shown to promote regeneration in the peripheral nervous system, we propose that ganglioside treatments might enhance regenerative growth but not lesion-induced sprouting.

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306.3 **CHARACTERIZATION OF A PROTEIN CAUSING A POSITIVE RESPONSE IN AN NGF RADIOIMMUNOASSAY.** T.L. Darling* (SPON: N.J. Pantazis). Lab. CNS Inj. and Regen., VA Med Center, and George Washington Univ. Med. Sch., Washington, D.C. 20422.

Nerve growth factor (NGF) activity is extracted from the submaxillary gland of Mastomys natalensis, an African rat, as a high molecular weight complex, termed 5S NGF, composed of beta NGF and NGF alpha-like proteins. When gland extracts were fractionated on Sephadex G-100, a minor peak detected by virtue of positive response in the competitive solid phase radioimmunoassay (RIA) for beta NGF eluted at 25-30,000 MW and contained 15-50% of the RIA activity recovered. Neurite regenerative biological activity generally was absent, but when present was low compared to the RIA measurements and could be blocked by anti-NGF antibody. The RIA activity was found to have a sedimentation coefficient of 2.5 S. When subjected to analytical isoelectric focusing in a sucrose density gradient, the RIA activity had an apparent pK of 4.5, similar to NGF alpha, and had no biological activity. The RIA activity of the G-100 pool was further purified and separated from residual biological activity by chromatography on DEAE BioGel. The DEAE fraction was found when analyzed by non-reducing denaturing SDS gel electrophoresis to contain 3 peptides: 24,000; 27,000; and 12,000 MW.

The similarity of the proteins purified from this fraction to mouse NGF alpha subunit and to Mastomys alpha-like protein suggested that the positive RIA response could be explained by binding of the radioactive NGF tracer to a protein of the 25,000 MW fraction. To test the hypothesis, ^{125}I-NGF was incubated with the DEAE purified material and then subjected to centrifugation through a sucrose density gradient. Tracer which had not been incubated with DEAE pool demonstrated poor recovery from the gradient and gave the expected sedimentation coefficient of 2.5S. Recovery of ^{125}I-NGF which had been incubated with the DEAE purified proteins was greater and two peaks were observed which had sedimentation coefficients of 3S and 4.5S. These results suggest that the RIA positive protein was similar to alpha NGF and binds mouse ^{125}I-NGF to give rise to a stable complex.
This research was supported by the Veterans Administration and by the NIH (#NS 04270).

306.4 VASOACTIVE INTESTINAL PEPTIDE INCREASES ACTIVITY-DEPENDENT NEURONAL SURVIVAL IN DEVELOPING SPINAL CORD CULTURES. D.E. Brenneman, L.E. Eiden*, and R.E. Siegel*. Lab. of Developmental Neurobiology, NICHD, Lab. of Cell Biology, NIMH,NIH, Bethesda, Maryland 20205

Vasoactive intestinal peptide (VIP) was investigated for possible trophic action on developing spinal cord neurons. The interaction of VIP with the electrical state of the cultures was also examined. Dissociated spinal cord-dorsal root ganglion (SC-DRG) cultures were prepared from 12-day old fetal mice. Blockade of spontaneous action potentials with tetrodotoxin (TTX) has been shown to produce significant decreases in cholinergic development and decreases in the total number of neurons. Vulnerability of spinal cord neurons to TTX treatment occurred during a critical period in development (day 7-21 in vitro). Previous studies also have shown that conditioned media obtained before or after the critical period produced increased neuron survival when applied to TTX-treated cultures which were within the critical period. The present study examines the possibility that VIP or a VIP-like substance is the agent in conditioned media which mediates activity-related trophic action.

VIP and/or TTX were added to cultures on day 9 in vitro. Neuronal cell counts were conducted after 5 days of treatment. Addition of 10^{-10} M VIP plus 1 μM TTX resulted in a 25% increase in neurons as compared to that of controls. TTX treatment alone produced a 20-25% decrease from controls. In cultures treated with VIP alone, a significant increase (20%) was observed from controls. Neuronal cell counts were also conducted on day 9, the beginning of the test period. The control cultures on day 9 were not significantly different from those observed with VIP plus TTX on day 14. The studies suggest that treatment with VIP on electrically inactive cultures protects from neuronal death which normally occurred in development and that which was TTX-mediated.

To test if VIP-containg neurons were present, VIP-like immunoreactivity (VIP-LI) was assayed for by RIA and immunofluorescent techniques. VIP content increased during development to a maximum on day 21 in culture. From 3-5% of the total neurons present in SC-DRG cultures were positive for VIP-LI.

Together these studies suggest that VIP is present in these cultures and that VIP may play a role in the competitive processes that influence activity-dependent neuronal survival in this model system.

306.5 LOCALIZATION OF NGF RECEPTORS ON CULTURED NEURAL CREST CELLS. P. Bernd. Dept. of Anatomy, Mount Sinai School of Medicine of The City University of New York, New York, N.Y. 10029.

Nerve growth factor (NGF) is required for the survival and maintenance of some neural crest derivatives, such as sympathetic and some sensory neurons. In order to gain insight into the role of NGF during early embryonic development, the appearance of cells bearing NGF receptors (possible targets of NGF) was studied in cultures of quail neural crest. Neural tubes (neural crest adherent) were removed from 48 h quail embryos, dissected free of somites and notochord (in 0.125% trypsin or 2.4 U/ml dispase in HBSS without Ca^{++} or Mg^{++}), and allowed to attach to gelatin-coated plastic tissue culture dishes containing medium (75% MEM, 15% heat inactivated horse serum or 15% fetal calf serum, 20 U/ml pen/strep, 0.12% sodium bicarbonate, 5% chick embryo extract). Within 24 h, the neural crest cells have migrated away from the neural tubes and the tubes were removed. After 3 to 5 days, cells were triturated and replated onto gelatin-coated glass coverslips. Incubation with ^{125}I-NGF (5 ng/ml; 1 h) was performed approximately 1 week after explantation following a 2 h rinse with chick embryo extract-free medium. Light microscopic radioautographic examination of cell cultures revealed heterogenous binding that was blocked by an excess of nonradioactive NGF (1.5 ug/ml), except for an apparent low affinity binding to debris-like aggregates. No binding was detected following incubation with inactivated ^{125}I-NGF, or in cultures prepared from somites or notochord. The pattern of ^{125}I-NGF binding was similar in medium containing either horse serum or fetal calf serum. Melanocytes did not appear to be labelled. It remains to be determined what cell types of those present in these cultures bind ^{125}I-NGF (i.e. adrenergic, cholinergic, serotonergic and somatostatin positive cells). In contrast, undifferentiated neural crest cells, still adherent to tube or 24 h following explantation, exhibited undetectable or very low levels of ^{125}I-NGF binding. In summary, preliminary studies have revealed specific ^{125}I-NGF binding to a heterogenous population of differentiating cells in neural crest cultures, while undifferentiated neural crest cells do not appear to bear NGF receptors. Supported by grant HD 17262 from NIH.

306.6 **REGULATION OF SYNTHESIS AND PROPERTIES OF NERVE GROWTH FACTOR AND ANTIBODIES TO NERVE GROWTH FACTOR.** K. Werrbach-Perez*, K. Hubner* and J. R. Perez-Polo, Dept. of Human Biological Chemistry & Genetics, The University of Texas Medical Branch, Galveston, Texas 77550-2777.

Nerve growth factor (NGF) is the name given to a family of proteins required for the survival, development and regeneration of mammalian sympathetic and sensory ganglia. Although similar in many respects, there are also differences among the species of NGF isolated to date that may have important consequences as to the regulation of NGF activity in vivo. NGF can be isolated as an alpha,betagamma$_2$ multimer from mouse submaxillary gland, a gammabeta complex from snake venom or an alphabeta complex from human term placenta. NGF of human origin, whether from term placenta or secreted by a human neurofibroma line, is antigenically indistinguishable; however, the murine beta-NGF is not recognized by antibodies to human beta-NGF and vice versa. NGF synthesized and secreted by rat glial and murine sarcoma cells is indistiguishable from murine beta-NGF either in terms of recognition by affinity purified antibodies to murine beta-NGF or of isoelectric point and molecular weight of the active molecular species. In particular, the mouse sarcoma line synthesizes the three different murine NGF subunits; alpha, beta and gamma. The amino acid composition, isoelectric point and molecular weight of human term placenta beta-NGF is very similar to that of murine beta-NGF. Using a modification of available in vitro immunization techniques, 12 hybridoma lines were developed that secrete antibodies directed to human beta-NGF. None of these antibodies crossreact with murine beta-NGF but two of the hybridoma secreted antibodies did crossreact with NTF-II, another neuronotrophic factor protein with a similar amino acid composition to human beta-NGF that has an acidic pI and is 70,000 daltons in size. Also, human term placenta beta-NGF can displace murine beta-NGF from NGF receptors on LAN-1 human neuroblastoma cells. These receptors are similar in kinetic and structural features to NGF receptors on chick sensory ganglia or PC12 cells. Supported by NIH NS18708 and The Robert A. Welch Foundation.

306.7 **CORRELATION OF NGF INDUCED DIFFERENTIATION WITH GANGLIOSIDE COMPOSITION IN THE HUMAN NEUROBLASTOMA LINE SK-N-SH-SY5Y.** J. R. Perez-Polo, K. Werrbach-Perez* and G. Rebel*†. (SPON: D. K. Rassin). Dept. of HBC&G., The University of Texas Medical Branch, Galveston, Texas 77550-2777; †CNRS, 67000 Strasbourg Cedex, France.

Nerve growth factor (NGF) is the name given to a family of proteins required for the survival and appropriate development of mammalian sensory and sympathetic neurons. Also, NGF has been demonstrated to display neuronotrophic effects on target neurons in vivo and in vitro. NGF-responsive human neuroblastoma cells SK-N-SH-SY5Y (SY5Y) will extrude neurites in vitro in response to NGF. Also, NGF accelerates rates of attachment of human neuroblastoma cells in culture. NGF-responsive human neuroblastoma cells have NGF receptors that display similar ligand binding kinetic properties when compared to chick embryonic sensory ganglia dissociated neurons. Also, it has been suggested that gangliosides can induce morphological changes in NGF-responsive neurons not unlike those observed with NGF. Here we wish to report that when an NGF-responsive human neuroblastoma clonal line (SY5Y) is exposed to NGF and allowed to differentiate, the ganglioside composition of its membranes is changed.

Gangliosides	Control(%)	NGF(%)	cAMP*%)
GM3	9.5	3.9	5.1
GM2	38.8	30.5	31.9
GM1	6.9	14.0	8.4
GD3	6.0	10.2	14.3
GD1a	36.2	39.1	37.8
GD1b	2.6	2.3	2.5
μg lipid sialic acid/mg	0.73	0.92	0.70

The use of dibutyryl cyclic AMP, previously shown to result in extrusion of neurites but not other aspects of differentiation unique to NGF-induced differentiation, also altered ganglioside composition in these cells. The significance of these changes in terms of the physiological effects of NGF on neuronal membranes as an important aspect of neurite elongation has not been determined. However, it would appear that treatment with dibutyryl cAMP does alter ganglioside content in a somewhat different manner than that observed for NGF treatment. Supported by NIH NS18708 and The Robert A. Welch Foundation.

306.8 GM1 GANGLIOSIDES STIMULATE NEURONAL REORGANIZATION AND BEHAVIORAL RECOVERY AFTER NIGRO-STRIATAL HEMITRANSECTIONS. AN HRP-STUDY. B.A. Sabel, G.L. Dunbar*, W.M. Butler* and D.G. Stein. Brain Res. Lab., Dept. Psychology, Clark University, Worcester, MA 01610.

GM1-ganglioside treatment has recently been shown to reduce behavioral deficits after unilateral and bilateral brain injury. In order to elucidate anatomical -morphological mechanisms that may account for these effects, adult, male rats were given partial hemitransections of the nigro-striato-nigral fibers and were treated with GM1 (Fidia Res. Lab., 30 mg/kg, IP) daily for 14 days. At various time intervals (3, 15 or 45 days) animals were killed, after receiving intrastriatal injections of horseradish peroxydase (WGA-HRP).

Behavioral results: Ipsiversive amphetamine-induced rotations were significantly reduced on days 2 and 14 in GM1-treated animals. These animals also rotated less after apomorphine injections on day 39. Anatomical results: Only a few HRP-labelled cells were seen in the ispsilateral substantia nigra pars compacta (iSNc) and ventral tegmental area (iVTA) on day 3 in both treatment groups. However, within 15 days, more labelled neurons were seen in iSNc ($p<.05$) and iVTA ($p<.07$) after GM1-treatment compared to saline controls. Comparable labelling was not observed in the saline group until day 45, when both groups had many labelled neurons in iSNc and iVTA. Sparse connections from the contralateral SNc (cSNc) did not degenerate in animals treated with GM1. In fact, their number was temporarily above that of saline treated animals ($p<.01$) and unoperated controls ($p<.05$). The absence of crossed connections in saline-treated rats indicates that they degenerate and do not re-appear until day 45.

These data indicate that, following unilateral, partial transections of the nigro-striato-nigral fibers, GM1 treatment accelerates reorganization of spared ipsilateral and interhemispheric nigro-striatal fibers (possibly via sprouting) and reduces both amphetamine- and apomorphine-induced rotational behavioral. While the reduction of amphetamine-induced rotations may be a correlate of neuronal reorganization, the reduction of ipsiversive rotations after apomorphine-challange may be related to the action of GM1 in preserving cell death in the striatum.

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306.9 GM1 GANGLIOSIDES REDUCE SPATIAL ALTERATION DEFICITS FOLLOWING BILATERAL LESIONS OF THE MEDIOFRONTAL CORTEX. G.L. Dunbar*, B.A. Sabel, A.C. Firl* and D.G. Stein Brain Res. Lab., Dept. of Psychology, Clark University, Worcester, MA 01610.

We have previously shown (Sabel, et.al., Science, in press) that administration of gangliosides reduce learning deficits on an escape/avoidance task following bilateral electrothermic lesions of the caudate nucleus. The present study was designed to determine whether ganglioside-induced reduction of behavioral impairments can be generalized to: (1) lesions in other areas of the brain; (2) injuries inflicted by different lesion methods; and (3) behavioral tasks which utilize a qualitatively different type of reinforcement paradigm.

Following bilateral aspiration lesions of the mediofrontal cortex, injections of GM1-gangliosides (Fidia Res. Lab., 30 mg/kg, IP) were given daily for two weeks to adult, male rats. After a 10-day postoperative recovery period, the animals were maintained on a 23 hr-50 min water deprivation schedule and trained to receive water reinforcement by solving a spatial alternation task in a T-maze. After one week of testing, the GM1-treated animals were statistically indistinguishable from sham-operated, saline-treated controls in the number of errors, number of perseverations, and the mean number of trials required to attain a criterion of 18 out of 20 correct responses. In contrast, rats with identical lesions and injections of saline were significantly impaired on these measures when compared with the sham-operated controls ($p<.05$, for all three measures).

In summary, our results demonstrate that ganglioside treatments attenuate spatial alternation deficits following bilateral aspiration lesions of the mediofrontal cortex. Taken together with our previous work, these findings provide evidence that gangliosides reduce behavioral impairments following brain injury, independent of specific lesion or testing parameters.

Supported by USAMRDC contract #DAMD 17-82-C-2205.

306.10 ACTIVITY OF A MUSCLE-DERIVED GROWTH FACTOR FOR SPINAL NEURONS IN VITRO AND IN VITRO. M.E. Gurney and B. Apatoff, Dept. Pharmacol. and Physiol. Sci., The University of Chicago, 947 E. 58th Street, Chicago, IL 60637.

Experimental antisera and sera from some patients with motor neuron disease (ALS) have previsously been shown to suppress terminal axonal sprouting at the neuromuscular junction (Nature 307:564-548). These sera reacted in common with a 56kd polypeptide present in conditioned medium from denervated rat diaphragm organ cultures. The 56kd antigen co-purifies on gel filtration (AcA44, Mr = 60-50kd) and chromatography over DE52 (elution with 0.15-0.25 M NaCl in 20mM NaHP04, pH7.5) with a survival activity for cultured spinal cord neurons (st24 chick neural tube). We now have produced a monoclonal antibody that demonstrates the survival activity is associated with the 56kd polypeptide. Rats were immunized with the DE52 fraction, and spleen cells were fused with mouse SP2/0 cells. Hybrids were initially screened by ELISA with the DE52 fraction. 1118 hybrids from 2 fusions yielded 188 positives by ELISA, 109 of these were rescreened by immunoblotting, and 11 were obtained that reacted with the 56kd antigen. At least one of these, B86.3 (an IgG2a) recognizes the receptor binding portion of the 56kd factor as the IgG2a blocks the factor's survival activity for cultured spinal cord neurons when added to the medium. The survival activity was retained on a B86.3-Affigel 10 column and the eluted material was found to be one-half maximally active in culture at 1 ng/ml or 2 x 10-11M. 50% suppression of terminal sprouting has been obtained in vivo by injection of the purified IgG2a. Thus, terminal sprouting is controlled in part by release of the 56kd factor from denervated or inactive muscle, and ALS has an auto-immune component directed against this factor. Whether or not the auto-antibodies against the 56kd survival factor produce the destruction of motor neurons seen in ALS remains unresolved. Supported by the ALSSOA, NALS, Searle Scholars Program, and Sloan Foundation.

306.11 TARGET-SIZE ANALYSIS OF A NEURITE ELONGATION FACTOR USING RADIATION INACTIVATION. M. D. Coughlin. Dept. of Neurosciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Neurite outgrowth from sympathetic and other peripheral neurons in cell culture is stimulated by a substrate-binding factor derived from medium conditioned over various classes of cells. The conditioned-medium factor (CMF) has been described in numerous systems, with activity being attributed to very high molecular weight material. Under non-dissociating conditions, CMF activity is eluted from a Sepharose CL-2B column in a large aggregate with an apparent molecular size of approximately 2 to 5 million (Coughlin, M.D., and Kessler, J.A., J. Neuro. Res.8:289, 1982). The active component has not yet been completely purified.

Gel electrophoresis of CMF partially purified by ion-exchange chromatography (Coughlin, M.D., et al., Dev. Biol. 82:56, 1981) demonstrates numerous protein bands. Treatment of the crude fraction with testicular hyaluronidase and gel filtration through a Sephadex G-100 column results in an active void volume fraction which produces a single major protein band at 220 kd on a gradient gel. However, the fraction is not completely homogeneous, as silver staining reveals numerous minor bands.

To define the functional size of the active molecule, partially purified CMF was subjected to radiation inactivation analysis. CMF was exposed to gamma irradiation over a dose range of 0.2 to 8 megarads. Inactivation of function is proportional to the size of the target molecule. Enzyme standards run simultaneously were used to construct a calibration plot. M_r of CMF was calculated to be approximately 200,000 by reference both to the calibration plot of standards and to the empirical formula derived by Kepner and Macey (BBA 163:188, 1968). Thus, radiation inactivation analysis of CMF activity suggests that the major electrophorestic band at 220 kd possesses the neurite elongation activity.

306.12 ANALYSIS OF VARIOUS CONCENTRATIONS OF SERUMS ON TISSUE CULTURE OF DISSOCIATED PRIMARY FETAL BRAIN STEM. M.L. Bonvicino* and E.C. Azmitia (SPON: I. Fand). Department of Biology, New York University, New York, NY 10003.

Brain stem of fetal rats (E 16-17) was minced in Hank's Balanced Salt Solution (HBSS, Gibco Labs.) containing 1% glucose. The tissue was then dissociated with Versene (Flow Labs.) and gentle repipetting and centrifuged for 20 minutes at 500 g. The supernatant was discarded and the cells were then resuspended in 5 ml of HBSS (without Ca^{++}, Mg^{++}) and centrifuged for 10 minutes. Approximately 4.5 ml of supernatant was discarded and the cells were resuspended in Neuronal Complete Media (no serum added) with a final plating density of 1.5-2 X 10^6 cells /cm^2. Various concentrations of Fetal Bovine Serum (FCS; 10%, 5%, 1%); Horse Serum (HS; 10%, 5%, 1%); Clex (Dextran Products; 10%, 5%, 1%, 0.1%); combinations of 10% FCS and 10% HS; 5% FCS and 5% HS and cells without serum were tested. The solution was plated on a collagen prepared Linbro Microtitration Plate (Flow Labs.) and incubated at 37°C, 4.8-5.2% CO_2 for 1 week. At least 3 separate experiments were performed in triplicate for each condition.

Amount of cell clustering, number of cells and processes were used as criteria to qualitatively evaluate the various serums tested. Results indicate that primary fetal brain stem neurons grow optimally in a concentration of 5% FCS. At 10% FCS there seemed to be less clustering, processes and fewer cells. Cells in 1% FCS showed less growth than at 10% FCS. Cells grown in 5% HS showed better growth than at 10% HS and 1% HS. A combination of 5% FCS and 5% HS showed better growth than the 10% FCS: 10% HS, and either, 10% FCS or 10% HS alone. Neurons grown in Clex 10% grew well, but not as well as 5% FCS. Cells grown in 5% and 1% Clex showed fewer processes and clustering. Cells in 0.1% Clex and in serum free media were dead within a day.

In order to quantitate protein synthesis in tissue culture, the incorporation of ^{35}S-methianine into precipitable protein was studied. Preliminary results suggest that the most incorporation took place in 5% FCS and 10% Clex. Lesser amounts were incorporated in cells grown in the other serums.

Our studies indicate that dissociated fetal brain stem cells seem to grow optimally in 5% FCS or 10% Clex as determined by cell morphology and protein synthesis.

This research supported by NSF-Grant BNS-83-04704.

306.13 BIOTINYLATED β-NERVE GROWTH FACTOR BINDS TO HIGH AFFINITY NGF RECEPTORS ON PC12 CELLS. M.B. Rosenberg*, E. Hawrot and X.O. Breakefield* (SPON: S.B. Edelstein). Depts. Human Genetics & Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

Chemically modified derivatives of β-nerve growth factor (NGF) can be used to study the binding and intracellular processing of NGF by cells bearing NGF receptors, as well as the structure/function relationships of this peptide hormone. NGF was biotinylated on carboxyl groups using biotin hydrazide and the coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. The reaction yielded an average ratio of 6 biotins per NGF dimer; a low level of covalently linked NGF dimers also occurred. The biotinylated NGF derivative (C-bio-NGF) was as effective as native NGF in competing with ^{125}I-NGF for binding to receptors on PC12 cells at 4°C. In contrast, NGF that was biotinylated on amino groups (N-bio-NGF) at a ratio of 2 biotins per dimer, using N-hydroxy-succinimidyl biotin, had only 45% of the binding activity of native NGF in competitive receptor assays at 4°C. Increasing the biotin:dimer ratio of N-bio-NGF to 4:1 further decreased receptor binding to 23% of native NGF.

C-bio-NGF, but not N-bio-NGF, was able to mediate the specific binding of ^{125}I-avidin to PC12 cells. A variant PC12 line lacking NGF receptors, PNR-18A (donated by M. Bothwell, Princeton) was not labeled by ^{125}I-avidin after prior incubation with C-bio-NGF. Biotinylated RNase A, which is similar to NGF in molecular weight and isoelectric point, did not mediate the binding of ^{125}I-avidin to PC12 cells. Binding of C-bio-NGF to PC12 cells, as detected by subsequent labeling with ^{125}I-avidin, was decreased by excess native NGF but not by RNase A, cytochrome C or insulin.

Experiments are underway to determine whether the biotinylated NGF derivatives are internalized and are biologically active. C-bio-NGF can be used to target lipsome-encapsulated DNA and drugs to cells bearing NGF receptors, including PC12 cells and melanomas, as well as to study NGF-receptor interactions and cellular processing using fluorescently labeled avidin.

306.14 RELATION OF HIPPOCAMPAL TROPHIC ACTIVITY TO CHOLINERGIC NERVE SPROUTING. A.M. Heacock, A.R. Schonfeld & R. Katzman, Dept. of Neurology, Albert Einstein Col. Med., Bronx, N.Y. 10461

The requirement of cultured neuronal cells for neurotrophic factors (NTF) has led to speculation about the function of such factors in CNS development and response to injury. Recently, stimulation of NTF production by injury to adult rat brain was found to maximize the survival of transplanted embryonic neurons (Nieto-Sampedro et al, J. Neurosci. 3:2219, 1983) and enhance sprouting of adult cholinergic axons into intrahippocampal iris implants (Schonfeld et al, Neurosci. Abst. 1983). Here we report further studies of the effect of manipulation of endogenous hippocampal NTF on the sprouting of septo-hippocampal fibers. Lesion of the entorhinal cortex (EC) is known to stimulate sprouting of septal axons into the hippocampus and has been reported to increase hippocampal NTF. In a further exploration of the connection between these observations, the effect of unilateral (right) EC lesion on hippocampal NTF (measured by 24h survival of chick ciliary ganglion cells) was compared with that on cholinergic sprouting (assessed by choline acetyltransferase (CAT) activity in iris tissue implanted into anterior hippocampus). CAT activity in irides implanted 3 weeks after right EC lesion and left in situ for 15 days was 2.5 fold higher than that from unlesioned controls (13.4±1.49 and 5.30±0.45 nmol/mg/h, respectively). At this time the ratio of right/left hippocampal NTF had increased 2-3 fold. If there is a cause and effect relationship between these two phenomena, then interference with the rise in NTF in EC-lesioned animals would be expected to alter iris CAT activity. A glial origin of the trophic factor has been suggested since treatments which increase NTF often result in gliosis (e.g., wounding, E.C. lesion or systemic kainic acid). Recently, methotrexate (MTX) treatment was reported to diminish this glial response (Avendano, Brain Res. 265:160, 1983). We therefore injected rats intraventricularly with 100 μg MTX at the time of EC lesion and measured hippocampal NTF after 5 weeks. While control animals (EC lesion alone) showed a right/left ratio of 2.23, in those injected with MTX, this ratio was only 1.44. Studies to determine the effect of MTX treatment on iris implant CAT activity are in progress. These results should aid in the elucidation of the origin and function of endogenous CNS trophic factors.

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306.15 **EFFECTS OF TUNICAMYCIN ON NERVE GROWTH FACTOR (NGF) BINDING AND NEURITE OUTGROWTH IN PC12 CELLS.** T. J. Baribault* and K. E. Neet*(SPON: M. Maguire). Dept. of Biochem., CWRU Med. School, Cleveland, OH 44106.

The rat pheochromocytoma-derived PC12 cell line was treated with tunicamycin (TM) in an attempt to define the role of N-linked carbohydrate in β-NGF binding to the specific cell surface glycoprotein receptor (NGFR). In addition to assaying the binding of 125-I-NGF to the rapidly and slowly dissociating classes of NGFR in PC12 cells (Landreth and Shooter, PNAS, 77: 4751, 1980), the neurite outgrowth response to NGF was measured by the dibutyryl cAMP co-incubation method (Gunning et al., J. Cell Biol., 89: 240, 1981) in both TM-treated and control cultures.

TM affected both the NGF binding and bioassays in PC12 cells in a dose and time dependent manner. Exposure to TM (1-10 μg/ml) for 24-36 hours prior to assay resulted in significant decreases in the rapidly dissociating component of NGF binding (30-50% of total specific binding at 100 pM NGF vs. 70% for controls) and in the proportion of PC12 cells capable of elaborating neurites in the presence of NGF and dibutyryl cAMP (20% of control response at 10 μg/ml TM). After a 36 hour drug exposure, cell viabilities ranged from 85-95% (by Trypan Blue exclusion) and total protein synthesis was 75% that of controls (based on 35-S-methionine incorporation). Thus the changes in binding and neurite outgrowth were probably due to underglycosylation of the NGFR in the presence of TM rather than a general decline in cellular metabolism.

The mechanism by which poorly glycosylated NGFR interacts with ligand and membrane components to yield these altered responses is unknown. However, Scatchard analyses over the NGF concentration range of 10 pM to 10 nM established that underglycosylation reduces the number of rapidly dissociating binding sites (50,000 vs. 215,000), does not change the total number of slowly dissociating sites and decreases the affinity of the slowly dissociating sites (4.4 nM vs. 2 nM). The decrease in percentage of cells responding in a companion neurite outgrowth assay suggests that the rapidly dissociating component of NGF binding is associated with neurite elaboration. The results with tunicamycin indicate that carbohydrate side chains are important for the function and/or accessibility of the NGF receptor in PC12 cells. (Supported by NIH grant NS17141 and NSF grant PCM 8314309.)

306.16 **ASSOCIATION KINETICS AND COOPERATIVE INTERACTIONS IN βNGF RECEPTOR BINDING TO PC12 CELLS.** N.R. Woodruff* and K.E. Neet* (SPON: S. Younkin). Dept. of Biochem., Case Western Reserve School of Medicine, Cleveland, OH 44106.

The association kinetics of ^{125}I-βNGF binding to the PC12 clonal cell line have been examined in detail at 0.5°, 20° and 37°. These data were examined utilizing a reversible second order integrated rate equation and the results were not consistent with a simple bimolecular process. At 37°, two independent association components were found to adequately explain the results. The faster component was estimated to have a second order association rate constant of $1.4(10^7)M^{-1}sec^{-1}$, while the rate constant for the slower component ($2.8(10^6)M^{-1}sec^{-1}$) was close to 5 fold lower.

The steady state binding results at 37° indicated only one class of binding sites (160,000±19,000 sites/cell) which had an apparent K_d of 0.5nM. One class of sites was also observed at 0.5° and the receptor concentration was found to be reduced (90,000±11,000 sites/cell) while the K_d was increased (1.6±.19nM). A significant level of positively cooperative interactions was observed at 37° which was not simply due to a failure to reach steady state conditions. Since cooperativity was never observed at 0.5°, a membrane event may be involved.

As shown by others, the temperature dependence of the dissociation kinetics indicated that while the rapidly dissociating component was only slightly slowed by lowering the chase temperature to 0.5°, the second component was slowed by about 230 fold, from $7(10^{-4})$±$1.5(10^{-4})sec^{-1}$ to $3.1(10^{-6})$±$0.14(10^{-6})sec^{-1}$. The binding parameters which describe the slowly dissociating component were investigated by utilizing this differential temperature dependence. The results from computer analysis using a nonlinear fitting program (LIGAND), suggest that this component consists of a single interacting class of about 2500 sites/cell which have a first stoichiometric steady state dissociation constant of 48pM and a second stoichiometric dissociation constant of 5pM, indicating positively cooperative interactions. The α, or the β subunit of NGF competed equally well with the fast and slow binding components with apparent EC50 values of 5μM and 200nM, respectively, in reasonable agreement with their K_d values for dissociation of the αβ and βγ complexes in solution.

The contribution of the two different classes of NGF receptors found on PC12 cells to the biological actions of NGF requires a clear understanding of their kinetic properties and their relationship to each other. Determining the molecular basis for these properties may hold significant clues to the mechanism of action of NGF. (Supported by Amer. Canc. Soc. Grant BC249.)

306.17 RESPONSES OF CULTURED RAT CHROMAFFIN CELLS TO NEURONOTROPHIC AND NEURITE PROMOTING FACTORS ARE DEVELOPMENTALLY REGULATED. K. Unsicker, S.D. Skaper and S. Varon. Dept. Biol. Sch. of Med., Univ. of Calif. San Diego, La Jolla, CA 92093

Cultured adrenal chromaffin cells from early postnatal rats have previously been shown to respond to neuronotrophic and neurite promoting factors by neurite growth and enhanced survival (Unsicker et al. PNAS 75:3498, 1978; PNAS 1984, in press; Doupe et al. Soc. Neurosci. Abst. 8:70.5, 1982; Doupe and Patterson Soc. Neurosci. Abst. 9:263.1, 1983). In the present study we have investigated the effects on cultured chromaffin cell (postnatal day - D1-D100) survival and neurite outgrowth of: NGF, ciliary neuronotrophic factor (CNTF), laminin (LN) and the polyornithine-binding neurite promoting factor from RN22 Schwannoma cells (PNPF). With no trophic factors the 4-day survival of chromaffin cells increased from 33% of the cells at D1 to 40% at D8 and 90-100% at D16 and older stages. At D1 NGF and CNTF had only a very small effect on survival after 4 days. At D8 NGF supported the survival of about 75% and CNTF of all chromaffin cells allowing the discrimination of three subsets of D8 chromaffin cells, one surviving without exogenous trophic factors, one only supported by CNTF and one supported by either NGF or CNTF.

Neurite outgrowth elicited by either NGF or CNTF amounted to 13-20% at D1, D8 and D16 and subsequently decreased to about 5-8% at D30 and virtually zero at D100. At this latter age both factors applied together clearly elicited neurites. Such a potentiating effect of NGF and CNTF on neurite growth was also seen at earlier postnatal ages. LN and PNPF did not stimulate neurite growth in the absence of trophic factors and, in their presence, did not enhance neurite recruitment as compared to a polyornithine substratum unless the cultures were carried for 7 days. Even at 4 days, however, LN and PNPF had clear and undistinguishable effects on neurite length and numbers of neurites and endings per cell. LN and PNPF combined with large doses of NGF did not initiate neurite growth from D100 cells. None of the above substrata affected chromaffin cell survival.

Our results suggest that chromaffin cells undergo age-related changes in their responses to neuronotrophic factors and extracellular matrix molecules.

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306.18 ASCORBIC ACID MEDIATES ACEYLCHOLINE RECEPTOR ACCUMULATION INDUCED BY BRAIN EXTRACT IN L_5 MYOGENIC CELLS. David Knaack* and Thomas Podleski* (SPON: Miriam M. Salpeter), Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853.

Addition of fetal calf brain extracts to cultured myogenic L_5 cells, leads to a 306% ± 35 (S.E.M., n=14) increase in acetylcholine receptor (AchR) as measured by ^{125}I-α bungarotoxin binding. Purification of the substance causing the major portion of this effect has been completed. The purified fraction induces an AchR increase on L_5 cells of 220% ± 71 (S.E.M., n=3). Amino acid analysis revealed no appreciable concentration of amino acids in the purified fraction. Nuclear magnetic resonance (NMR) spectra confirmed the absence of peptide bonds. The major NMR resonances at 3.73, 4.00 and 4.50 ppm (relative to DSS), and their respective multiplicities are indicative of ascorbic acid. Gas chromatography-mass spectra of the silated purified fraction were identical to tetra-silated ascorbic acid. The ultraviolet spectrum at neutral pH contained a single absorption maximum at 265 nm which shifted to 243 nm upon acidification, characteristic of ascorbic acid. The ascorbic acid extinction coefficient was used to calculate a yield of 100-300 ug of ascorbate purified from 1 g. wet weight of fetal calf brain. This value is consistent with published values of 100-500 ug ascorbic acid per gram wet weight of vertebrate brain tissue.

Commercial ascorbic acid, when added to L_5 cells at media concentrations of 6 ug/ml, induced a maximal AchR increase of 248% ± 37 (S.E.M., n=6). These data demonstrate that the presence of ascorbic acid in aqueous extracts of brain is sufficient to account for their ability to induce AchR accumulation in L_5 myogenic cells. Supported by NIH Grant NS14679 and NIH Predoctoral Training Grant GM07469.

306.19 LOCALIZED PROTEOLYSIS REGULATES NEURONAL-GLIAL CELL INTERACTION IN DEVELOPING NERVOUS SYSTEM IN CELL CULTURE. N. Kalderon, The Rockefeller Univ., New York, NY 10021.

The role of the localized extracellular proteolysis in tissue remodelling, i.e., the breakdown and construction of cellular interactions during histogenesis of the nervous system is being studied. Dissociated chick embryonic spinal cord cells maintained in a chemically-defined medium serve as a model system for this study. It was previously established that these cells produce extracellular plasminogen activator, and that when purified plasminogen is added to this medium, plasmin is generated and its proteolytic activity acts as a mitogen inducing glial cell division in this spinal cord cell population (Kalderon, 1982; J. Neurosci. Res. 18:509.

It is reported in the following that localized extracellular proteolysis, i.e., the plasmin-generating system, promotes neuronal-glial physical cell contact. In studies of [^{3}H]thymidine uptake into the cells, as a response to addition of plasminogen and which were developed for autoradiography, only those cells closely associated with neurons or neuronal processes were labeled with [^{3}H]thymidine, i.e., were dividing. If proteolysis were the primary mitogenic signal for the glial cells, the radiolabeled cells would have been randomly spread in the dish. However, the results clearly indicate that glial cells cannot divide unless they are in close physical association with neurons. Furthermore, since the net effect of the proteolytic activity was an increase in the rate of glial cell division, and since all the dividing cells were associated with neurons, it is assumed that the proteolysis promotes proper neuronal-glial interactions. Specifically, it is postulated that proteolysis cleaves one or more cell surface proteins and that only these protein fragments can mediate the specific neuronal-glial cell interactions. It is further hypothesized that once neuronal-glial cell interaction is established, some neuronal component dictates glial cell division.

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306.20 RAPID EFFECTS OF NERVE GROWTH FACTOR (NGF) ON SURFACE MORPHOLOGY AND ACTIN DISTRIBUTION OF PC-12 CELLS. K. Morrison-Graham, S. Huttner* and P. O'Lague. Dept Biology and Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles, CA

Polypeptide hormones, including insulin, epidermal growth factor (EGF), and platelet-derived growth factor, produce in a variety of cells surface membrane ruffling, usually within minutes. Similarly, NGF has been found to induce rapid surface changes in the neuron-like clone PC-12 (Connolly et al, J Cell Biol, 82:820, 1979). Here we present evidence that NGF triggers these changes locally and that this involves specific cytoskeletal elements.

Multinucleate PC-12 cells, produced by chemical fusion, were used to follow the sequence of events in single identified cells in the phase contrast microscope. NGF (β; 1-1000 ng/ml) caused two stages of transient membrane activity: First, ruffles appeared on the dorsal surface of the cells within 30 sec and continued for 3-4 min; second, as the dorsal ruffles subsided large ruffles appeared along the entire cell perimeter. By 10 min these also disappeared. Concurrently with these changes a general spreading of the cells occurred, creating an increase (up to 45%) in substrate contacted surface area within 10 min. These responses were blocked by anti-NGF antibodies. Ruffling responses could be limited to one region of the cell surface by locally applying NGF (1 μg/ml) with a micropipette (5 μm tip dia). When the pipette was directly above a small portion of the cell surface only that region underwent ruffling (followed up to 30 min).

Indirect immunocytochemistry was used to determine cytoskeletal involvement. Use of anti-actin antibodies revealed a transient change in the distribution of actin following NGF. Untreated cells were diffusely stained. However cells treated with NGF showed regions of intense staining at sites of membrane ruffling. Tubulin antibody staining patterns were not affected. Furthermore, cytochalasin B (10^{-6}M) but not colchicine (10^{-6}M) dramatically decreased the NGF-induced ruffling.

EGF and insulin produced somewhat different ruffling responses in PC-12 cells. Unlike insulin and NGF, the EGF response was concentration dependent. EGF and insulin also triggered a transient change in actin distribution.

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306.21 NEUROTROPHIC FACTOR: PARTIAL PURIFICATION OF A FACTOR FROM SHEEP SCIATIC NERVE WITH TROPHIC EFFECTS ON DENERVATED MUSCLES OF RATS. H.L. Davis, E.A. Heinicke*, J.A. Kiernan and R.A. Cook*. Departments of Biochemistry and Anatomy, The University of Western Ontario, London, Ontario, N6A 5C1, Canada.

Atrophy in a denervated muscle results from the disuse caused by paralysis of the muscle, and from the loss of special neurotrophic substances. Crude extracts of protein from rats' or sheep's sciatic nerves have been shown to prevent the non-disuse atrophy of rats' extensor digitorum longus (EDL) muscles denervated for 7 days when administered by daily intramuscular injections.

Crude extracts of sheep's sciatic nerves were fractionated by gel-liquid chromatography. After each step of purification, the neurotrophic activities of the various fractions of extract were assessed on denervated rats' EDL muscles. The degree of atrophy was assessed to determine which fraction contained the active principle. Affinity chromatography on concanavalin A-Sepharose revealed that the trophic substance was adsorbed to the con-A and thus is probably a glycoprotein. Further fractionation by gel filtration on Sephadex G-100 revealed that the active substance has a molecular weight of approximately 100,000. Purification by ion-exchange column chromatography on DEAE cellulose produced an active fraction containing substances with Pi's between 6.4 and 6.6, as determined by polyacrylamide gel isoelectric focusing. This active fraction produced three bands on sodium dodecyl sulfate-gel electrophoresis which had apparant molecular weights between 70,800 and 141,000. Antibodies will be raised to these three proteins and the purification will be completed by immunoaffinity chromatography.

Thus the trophic substance is an acidic glycoprotein of approximately 100,000 daltons. At this point a 250 fold purification has been acheived.

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306.22 EFFECTS OF POLYPEPTIDE GROWTH FACTORS ON RAT CULTURED MUSCLE. V. Askanas and S. Cave*. Neuromuscular Center, USC School of Medicine, Los Angeles, CA 90017.

We have previously shown that combined continuous addition to the culture medium of fibroblast growth factor (FGF), epidermal growth factor (EGF) and insulin (I) has a beneficial synergistic influence on human muscle development in tissue culture (Soc Neurosci Abstr, 9:2, 836, 1983). We have now examined the influence of those peptides on primary cultures of embryonic rat muscle in 5 experiments. Muscle cultures were established with trypsin-dissociated cells from thigh muscle of 18-19 day rat embryos, in 60 mm Petri dishes containing $5x10^{5}$ cells. Control medium (CM) consisted of 67% Dulbecco's MEM (Gibco), 22% Medium 199 (Gibco), 10% horse serum, and 1% antibiotics. Experimental medium (EM) consisted of CM plus the combined addition of 10 ug/ml insulin, 50 ng/ml FGF, and 10 ng/ml EGF. Cultures were examined morphologically daily by phase-contrast inverted microscopy. Biochemical assays at days 8, 15, and 22 were for total acetylcholine receptors (AChR) by ^{125}I alpha-bungarotoxin binding, creatine kinase activity (CK), and protein, each assay expressed per dish. From day 1-8 there was no morphologic difference between cultures grown in CM and EM. At day 8, AChR in EM-cultured muscle was 96% that of CM muscle. However, CK of EM cultures was 137% that of CM cultures. At day 15, EM-cultured muscle had thicker and better-developed muscle fibers; total AChR was 220% and CK activity 227% that of CM-cultures. Comparing day 15 to day 8, total AChR in EM cultures increased 347%, but in CM cultures increased only 97%. In day-15 cultures compared to day-8 cultures, CK increased 1135% in EM cultures, but only 648% in CM cultures. At day 22 there were only slight morphologic differences between the two sets of cultures. Total AChR in EM cultures was 253% that of CM cultures, whereas CK activity of EM cultures was only 158% that of CM cultures. At 22 days, in EM cultures, AChR was increased 124% compared to day 8; however, in day-22 CM cultures AChR was decreased 15% compared to day 8 (day-22 AChR in EM was 253% that of day-22 CM). Protein values at all points examined were virtually the same in the two sets of cultures.

Our studies indicate that FGF, EGF, and insulin together have a beneficial influence on rat muscle in long-term culture. This influence: 1) is specific to muscle cells in this mixed cell culture system since it enhanced AChR and CK, while total protein value remained unchanged; 2) is not related to mitogenic properties of the growth factors since it was not evident during the first 8 days of growth; 3) is possibly a neurotrophic-like "early-maturation factor" since it affects initial maintenance (decreased degradation? increased synthesis?) of AChR. (Supported by a grant from Muscular Dystrophy Association.)

307.1 Motor neuron size changes depend upon integrity of axonal pathways to target muscles in transforming shrimp claws. DeForest Mellon, Jr., Department of Biology, Univ of Virginia, Charlottesville, VA 22901.

In the snapping shrimp, Alpheus, the major chelipeds are dimorphic, and the motor neurons that supply the claw closer muscles exhibit size asymmetries that are correlated with target volumes. Closer motor neurons in the snapper are as much as 50% larger than homologous neurons to the pincer claw. Furthermore, snapper closer muscle excitatory junctional potentials exhibit more facilitation than do junctions in the pincer, and mean quantal output of snapper closer excitatory nerve terminals is 2-3 times that of pincer closer terminals. During growth of identified muscle fibers in crayfish motor nerve properties are partially dependent upon target muscle growth [Lnenicka and Mellon, J. Physiol., 345 (1983) 285]. During transformation of a pincer to a snapper claw in shrimp, the neuron anatomical and functional asymetries are reversed. To what extent do these modifications in properties directly depend upon changes in their targets? We sought to answer this question by interrupting the axonal pathways between motor neuron cell bodies and target muscle fibers. Pincer claw transformation was initiated by snapper removal and on the same day the limb nerve containing closer motor axons was ligated and sectioned in the pincer meropoite. In normal or control animals one moult cycle into transformation motor neuron soma size reversal is 50%-90% complete. In animals in which the relevant limb nerve was surgically sectioned, however, cell size reversal was not evident and the relative soma dimensions resembled those in control groups [control groups consisted of (1) animals in which the secondary limb nerve was severed in transforming claws and (2) non-transforming animals in which the relevant limb nerve of both pincer and snapper claws were sectioned.] These techniques cannot differentiate between failure of cell size reversal due to axonal damage per se and failure due to interuption of an important information pathway. Thus we have started experiments in which pincer claw transformation is initiated by crushing the snapper limb nerves [Mellon & Stephens, Nature, 272 (1978) 246]. Regeneration of snapper closer motor axons should, in this case, reestablish motor nerve-muscle communication and may prevent the reduction in snapper closer neuron size which usually accompanies transformation following snapper removal. This result would provide additional evidence for trophic dependency of neuron cell size upon target properties. Supported by USPHS Research Grant NS-15006.

307.2 RECEPTOR-MEDIATED UPTAKE OF ^{125}I-TRANSFERRIN BY EMBRYONIC CHICKEN DORSAL ROOT GANGLION NEURONS IN CULTURE. G. J. Markelonis, T. H. Oh and P. Azari*. Dept. Anatomy, Univ. Maryland School of Medicine, Baltimore, Maryland 21201.

Transferrin is a growth-promoting plasma protein which is known to occur within developing neurons. Since little information exists on the process by which transferrin is internalized by neurons, we studied this process using dissociated embryonic chicken dorsal root ganglion neurons in culture. Cultured dorsal root ganglion neurons were incubated in the presence of 3.75 nM ^{125}I-transferrin at 37°C, the cultures were extensively washed, the neurons were solubilized in a Triton X-100-containing buffer and internalized ^{125}I-transferrin was quantified with a gamma counter. ^{125}I-transferrin was internalized in a linear fashion for at least 60 min, and this uptake was abolished by the presence of 1.25 µM unlabeled transferrin. No competition for the uptake of ^{125}I-transferrin was observed in the presence of 1.25 µM ovalbumin, cytochrome c, hemoglobin, insulin, horseradish peroxidase, aldolase or the carboxyl-terminal fragment ("half-site") of transferrin. By contrast, uptake was inhibited by approximately 50% in the presence of the amino-terminal fragment ("half-site") of transferrin (1.25 µM) or in the presence of concanavalin A (1.25 µM). The presence of internalized ^{125}I-transferrin within neurons was confirmed by autoradiography at the light microscopic level. Furthermore, the transferrin receptors were visualized immunocytochemically on the surface membranes of dorsal root ganglion neurons using rabbit antibodies directed against transferrin receptors from chicken reticulocytes. From these data, we conclude that transferrin is internalized by neurons via receptor-mediated endocytosis, and suggest that this protein may serve an important role in the development and survival of dorsal root ganglion neurons.

Supported by the NIH (NS 16076-GJM and NS 15013-THO), the MDA (THO) and the Frank C. Bressler Research Fund of the University of Maryland School of Medicine (GJM).

307.3 PHOTORECEPTOR DIFFERENTIATION IN CHICK EMBRYO NEURAL RETINA MONOLAYER CULTURES. James D. Lindsey, Cynthia L. Elsner* and Ruben Adler. The Wilmer Institute, The Johns Hopkins University, Baltimore, Maryland 21205.

There is almost no information regarding molecular factors controlling photoreceptor survival and differentiation. This deficit is largely due to the unavailability of adequate culture systems for their investigation. We report here that many cells present in glial-free retinal monolayer cultures express photoreceptor structural and biochemical properties in vitro.

Neural retinas from 8-day chick embryos, a time when photoreceptor cells are already postmitotic but still largely undifferentiated, were dissociated and cultured for 3-7 days as described by Adler et al. (Devel. Neurosci., 1982) with some modifications.

Monopolar cells, which were among the most abundant neurons in these cultures, showed several morphological features characteristic of developing chick cones. Overall, the organization of these cultured cone-like cells appeared highly polarized with a single short, unbranched neurite at one pole. The region of the cell body closer to the neurite was almost completely occupied by the nucleus. More distally, the cells showed ultrastructural characteristic inner segment elements, including: i) a large, pigment-containing vacuole similar to the lipid droplet present in chick cones; ii) a cluster of mitochondria; iii) a polarized Golgi apparatus; and on occasion iv) a paraboloid. A cilium was also evident in some sections. Cultured cone cells could be selectively stained with fluorescent peanut lectin. This lectin has been shown to be specific for chick cones in vivo (Blanks and Johnson, in press). Staining of cone-like cells, which can be inhibited by the specific sugar β-galactose, was strongest in the inner segment region and in a membranous expansion originating at the distal end of the inner segment. Other neurons present in the cultures bound concanavalin A but not peanut lectin. The distal membranous expansion was seen by scanning electron microscopy to be highly flattened and to express many filopodia that appeared to be strongly attached to the substratum.

The degree of cone cell differentiation following 6 days in vitro, including the lack of outer segment development, is similar to that observed in retinas allowed to develop for 14 days in ovo. These cultures thus appear as an attractive experimental system to investigate the regulation of photoreceptor survival and differentiation. The influence of pigment epithelium-derived molecules on these phenomena is now being investigated in this laboratory.

Supported by NIH Grant EY 04859.

307.4 ACETYLCHOLINE- AND DOPAMINE-INDUCED EXCITATION OF CULTURED NEWBORN RABBIT NODOSE GANGLION NEURONS: EFFECTS OF CO-CULTURE WITH CAROTID BODY FRAGMENTS. W.F. Goldman*, M. Sato*, L. Stensaas and C. Eyzaguirre. Dept. of Physiology, Univ. of Utah, Salt Lake City, UT 84108.

We are studying the effects of co-culturing nodose ganglion neurons along with fragments of carotid body (diam. 100 µm) on the expression of acetylcholine (ACh) and dopamine (DA) receptors on the neurons. Nodose ganglia were obtained from newborn rabbits, enzymatically dissociated, and a suspension of dissociated cells was plated and maintained in Eagle's minimum essential medium containing NGF 7S (1 µg/ml), 5% fetal bovine and 5% adult rabbit sera, and insulin (5 µg/ml). Two days after plating, cytosiine arabinoside was added to retard proliferation of non-neuronal cells. However, some fibroblasts and glia were always present. Electrophysiological studies were made 18-21 days after plating. At this time neuronal diameters had reached 35-45 µm and extensive networks of processes had developed. For those neurons grown with carotid body fragments there appeared to be preferential growth into the fragment and some synapses on glomus cells were observed. The neuronal membrane potentials (MP) generally ranged from -50 to -70 mV although potentials as high as -84 mV were observed. Small puffs of ACh (10^{-6}M-10^{-3}M) pressure ejected onto the somas from micropipettes (tip diameter 10-20 µ) resulted in rapid depolarizations of 5 to 14 mV depending on tip diameter and ejection pressure and duration. In those instances where threshold was reached, a brief train of action potentials was elicited. This response was observed in almost all neurons studied whether cultured alone or with carotid body fragments, and the response was blocked by hexamethonium (10^{-4}M). Less than 10% of the neurons in either group were insensitive to ACh. Of the neurons cultured alone, 48% were also sensitive to dopamine (DA; 10^{-5}M to 10^{-3}M). Like ACh, DA elicited a depolarization (4-16 mV) and a train of action potentials if threshold was reached, which were blocked by haliperidol (10^{-6}M). In contrast, there were significantly fewer (12%) DA-sensitive neurons among those cultured along with carotid body fragments. Further, the magnitude of the responses of susceptible cells (3-10 mV) was diminished and usually action potentials could not be induced. These data suggest that carotid body cells may influence the expression of dopamine receptors in these sensory neurons. (Supported in part by NIH grant No. 5 R01 NS05666.)

307.5 LOCALIZED UPTAKE OF 2-DEOXYGLUCOSE BY THE LATERAL MOTOR COLUMN OF SPINAL CORD EXPLANTS AS A FUNCTION OF TARGET TISSUE AND SUBSTRATUM. W.L. Muhlach* and E.D. Pollack. Inst. Study of Develop. Disabil. and Dept. Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL 60608.

We have previously demonstrated an in vitro tissue interaction between developing tadpole spinal cord neurons and their mesenchymal hind-limb target (J. Neurosci. Res., 8: 343, '82). This interaction involves target tissue control, via release of a diffusible factor, of direction and quantity of neurite growth, neurite growth rate characteristics, neuronal survival and neurotransmitter-based maturation of neurons. These processes parallel in vivo developmental events. Neuronal growth parameters are similarly influenced by the artificial attachment substratum, poly-DL-lysine (PLYS), that mimics the effect of the target.

To further characterize this developmental interaction, the metabolic activity of several spinal cord regions was assayed under conditions related to target tissue and attachment substratum. Autoradiographic analysis of cord explants following a one hour incubation in 2-[1,2-^{3}H] deoxy-d-glucose provided a metabolic activity index of cord regibns (grain density/area) with respect to experimental variables. Grain counts, standardized for background and specific activity of incubation medium, were made of spinal cord marginal zone, lateral motor columns (LMC), non-motor intermediate zone and ventricular zone. Only the LMC region of the spinal cord showed significant increases in metabolic rate as a function of the target tissue or the substratum. Grain counts of marginal, non-motor intermediate and ventricular zones were constant regardless of culturing conditions. The LMC of spinal cord explants co-cultured with limb mesenchyme on collagen substratum showed an increase of 36% over those of cords cultured on collagen. Target tissue co-cultured with cord explants on PLYS did not elicit an increase in grain density beyond that resulting from PLYS alone. Since target and substratum effects do not appear to be additive they may operate through a similar mechanism. It is concluded that this action of the target is specifically directed toward the LMC neuron population of spinal cord. A substratum-factor-neuron membrane interaction resulting in increased metabolic activity may be able to explain some aspects of target- and substratum-based neuronal growth regulation.

307.6 REGULATION OF NERVE GROWTH FACTOR SYNTHESIS IN TARGET TISSUES OF THE PERIPHERAL AND CENTRAL NERVOUS SYSTEM. S. Korsching and H. Thoenen, Max-Planck-Institute for Psychiatry, Dept. of Neurochemistry, 8033 Martinsried,FRG

Nerve growth factor (NGF) is not only essential for the development and maintenance of function and structure of the peripheral sympathetic and sensory nervous systems, but may also exert regulatory influences on central cholinergic neurons. For the analysis of NGF levels in vivo, we have developed a sensitive two-site enzyme immunoassay which can detect 0.01 fmole NGF/assay. Using this assay we determined the NGF content of rat sympathetic ganglia (19-25 ng NGF/g wet weight) and sympathetically innervated organs (0.3-2.1 ng NGF/g wet weight). The NGF content of the target organs was found to be correlated with the density of their sympathetic innervation (Proc. Natl.Acad.Sci 80, 3513 (1983). The asymmetric accumulation of NGF distal to a crush of the rat sciatic nerve provided evidence for the retrograde axonal transport of endogenous NGF (Neurosci. Lett. 39, 1 (1983).

Treatment of rats with 100 mg of 6-OH-dopamine/kg body weight, which selectively destroys the sympathetic nerve terminals, led to a 2-4 fold increase in the NGF levels in sympathetically innervated target tissues (iris, submandibular gland, heart) within 24 h. Conversely, during this time period, the NGF content of the sympathetic ganglia decreased to 3% of control values. Similar results were obtained when axonal transport was blocked with colchicine. These data suggest that NGF synthesis is confined to the innervated target organs and does not occur in ganglionic nonneuronal cells. The contribution of the different cell types to NGF synthesis has been investigated in cultures of dissociated rat iris, a densely innervated target organ. There, NGF synthesis was studied after complement-mediated killing of specific cells using antibodies against cell type-specific surface antigens. These studies demonstrated that Schwann cells do not significantly contribute to NGF synthesis in this organ.

In analogy to the situation in the periphery, we also found a region-specific distribution of NGF in the rat brain. The highest values were found in areas with dense cholinergic innervation, such as frontal cortex and hippocampus. The response of the hippocampal NGF content to cholinergic denervation by septohippocampal lesions is currently under investigation.

307.7 EVIDENCE FOR TRANSSYNAPTIC REGULATION OF SYNAPTIC VESICLE AND NEURONAL CELL SURFACE ANTIGENS IN RAT SUPERIOR CERVICAL GANGLION K.F. Greif and H.I. Trenchard*, Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010

Previous studies have shown that the normal development of a synaptic vesicle membrane protein (SV) and a neuronal cell surface heparan sulfate proteoglycan (HeS-PG) in the rat superior cervical ganglion closely parallels postnatal increases in neurotransmitter synthetic enzymes (Greif and Reichardt, J. neurosci. 2:843, 1982). The current study examined whether preganglionic input is required for the normal maturation of these antigens. Monoclonal antibodies were used to monitor changes in antigen expression. One antibody (SV 48) recognizes a 65kD integral membrane protein associated with synaptic vesicles. Two antibodies bind HeS-PG: PG 22 binds to a determinant on the core protein and PG 3 binds to a determinant on the HeS side chain. The cervical sympathetic trunk was cut bilaterally in neonatal or adult rats, with littermates serving as unoperated controls. At selected times after surgery, rats were sacrificed and SCGs were removed and pooled. Levels of antigen were determined by RIA. Antigen localization was carried out using immunocytochemical methods.

Following neonatal deafferentation, total protein per ganglion was slightly reduced. SV levels were reduced at 7, 14, and 30 days, consistent with the loss of presynaptic terminals. At 30 days, SV was present at 24% of control levels. Since SV is found in principal ganglion neuron cytoplasm as well as in terminals, this result suggests that synthesis is reduced after neonatal deafferentation. Such a reduction is not detected by immunocytochemical staining. In contrast, adult deafferentation appears to increase SV levels, at least transiently. Further studies are in progress to investigate this observation. Expression of the core protein of HeS-PG was not affected by deafferentation in neonatal or adult rats. Levels of HeS side-chain were significantly reduced at 14 and 30 days postnatal, while adult surgery had no effect. These results suggest that processing of HeS-PG side chains by principal neurons is partially regulated by innervation.

These studies indicate that transsynaptic regulation of synthesis and processing of neuronal molecules may be a more general feature of development than previously thought.

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307.8 THE EFFECTS OF CONDITIONED MEDIA FROM PERIPHERAL TARGET TISSUES ON DEVELOPING SENSORY NEURONS IN TISSUE CULTURE, A.E. Cole, G.G. Chen*, A.B. MacDermott, and J.L. Barker, Lab of Neurophysiology, NINCDS, NIH, Bethesda, MD 20205.

Dorsal root ganglion (DRG) cells grown in culture characteristically show single spike firing and rectification when the membrane potential is depolarized and anomalous rectification when hyperpolarized. Typically, these cells have been grown in co-culture with central neurons or isolated in monoculture. Therefore, we have investigated the effects of conditioned media (CM) from peripheral target tissues grown in culture on the physiology of developing DRGs. The DRGs were dissected from 13 day old fetal mice, dissociated and plated on collagen-coated plastic dishes. The cells from each dissection were separated into control and experimental groups and maintained for 1-11 weeks under appropriate conditions. CM from explanted skin, skeletal muscle, and gut cultures or from dissociated skeletal muscle cultures was applied to the cultures of DRGs twice weekly. Membrane properties were recorded intracellularly with conventional, high resistance electrodes and low resistance patch electrodes. The bathing medium was Hank's salt solution with 2 mM Ca, 2 mM Mg, 10 mM HEPES, and 6 mM glucose. Control properties of DRG neurons were established by recording from 71 cells of various ages. 28 additional control cells were studied from dissections with paired experimental groups. Averaging over all ages of control cells, we found that the resting membrane potential (RMP) was -56.5 ± 5.6 mV (99), the membrane time constant (τ) was 3.8 ± 1.3 msec (14) and the input resistance (R_I) was 30.2 ± 12.8 M Ohms (70) (mean $\pm$ s.d.,(n)). Under control conditions, 2% of the cells showed repetitive firing behavior during sustained depolarizing current. When cells grown in CM were studied, the RMP, τ, and R_I were not significantly different. However, the percentage of the cells showing repetitive firing behavior was increased to 26 %. Results measured with patch electrodes showed an even greater increase in the percentage of conditioned cells showing repetitive firing. Since the passive membrane properties under study were unchanged by the CM while the percentage of repetitively firing cells was increased, we conclude that the effects of the CM may be on one or several active membrane properties. This action could be directly on the DRGs or indirectly through effects on the background cells.

307.9 CHICK EYE EXTRACT INCREASES CHOLINERGIC ENZYME ACTIVITY IN SYMPATHETIC GANGLIA IN CULTURE.
L. Iacovitti, T.H. Joh, D.J. Reis and G. Teitelman. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

In rat sympathetic neurons the expression of not only adrenergic but also cholinergic properties can be influenced by certain factors in culture (Bunge et al., Science 199:1409-14-16, 1978; Patterson et al., Sci. Amer. 239:50-59, 1978). In chicks, one such factor found in embryonic eye extract (EEE) has been shown to increase the activity of the cholinergic enzyme choline acetyltransferase (CAT) in cultures of parasympathetic neurons of chick ciliary ganglia (Varon et al., Brain Res. 173:29-45, 1979; Nishi and Berg, J. Neurosci. 1:505-513, 1981). In the present study, we sought to determine whether in culture a) chick sympathetic neurons express cholinergic as well as adrenergic properties and, b) if so, whether EEE in these cultures will increase CAT activity. To address these questions, lumbar and sacral sympathetic ganglia from embryonic day (E) 13-14 chick were dissociated and plated on collagen-coated dishes. Four groups were established and maintained on feed supplemented with either fetal calf serum (group 1); fetal calf serum and EEE from E15 chick (group 2); horse serum (group 3) or, horse serum and EEE (group 4). All feeds contained 100ng/ml of 2.5 S NGF. We found that only those cultures supplemented with EEE (Groups 2 and 4) developed detectable levels of CAT enzyme activity (0.066 ± 0.003 nmoles acetylCoA/ganglion/hr; n=7) at 2-4 days and (0.186 ± 0.012 nmoles acetylCoA/ganglion/hr; n=6) at 12-14 days in vitro. In order to determine whether the survival of different populations of cells within the four feed groups was responsible for these differences in enzyme activity, cultures were incubated with specific antibodies to the adrenergic enzyme, tyrosine hydroxylase (TH) and processed for immunocytochemistry. Irrespective of nutrient medium, all neurons in all cultures were TH-immunoreactive. Moreover, neurons in all feed groups were able to take up and store exogenously administered ^{3}H-norepinephrine (1uM) in the absence, but not in the presence of the specific uptake inhibitor desmethylimipramine (10uM).

This study indicates that under certain conditions in culture a) chick sympathetic neurons express both adrenergic and cholinergic properties; b) at least a subpopulation of these cells simultaneously express traits of both phenotypes; and, c) factor(s) present in EEE modulate the expression of at least the cholinergic enzyme CAT.

307.10 TARGET INFLUENCES UPON TERMINAL MORPHOLOGY AND ACH SYNTHESIS IN CULTURED CHICK CILIARY GANGLION NEURONS. D. Bruce Gray and Jeremy B. Tuttle, Phsyiology Section, The Univ. of Connecticut, Storrs, CT 06268.

We have shown that co-culture of CG neurons with pectoral muscle or on myotube membrane remnants increases both basal acetylcholine (ACh) synthesis and depolarization stimulated synthesis over that of neurons cultured alone. Thus, neuronal contact with the target tissue membrane influences transmitter synthetic capacity and its responsiveness to transmitter depletion.

The rate limiting step of ACh synthesis in culture is a sodium-dependent-high-affinity choline uptake (SDHACU) system similar to that present in CG terminals in the iris. The neuronal soma in vivo lacks SDHACU. The time course of accumulation, apparent Km and sodium dependency remain constant in all culture conditions. Although choline acetyltransferase levels per neuron increase in co-culture (100% on myotubes, 33% on myotube remnants) the absolute values are 2-3 orders of magnitude greater than required for the observed synthesis rates.

Morphologically, CG neurons on myotubes differ from neurons plated alone by the formation of specialized terminal contacts as revealed by Lucifer Yellow injection. A stereotypic cluster of large terminal swellings of neuronal processes was seen on the surface of many myotubes, resembling a neuromuscular synapse. Functional NMJ's are common in these cultures. These specialized structures were also seen in cultures on lysed myotube remnants but were never observed in cultures of neurons alone, despite the formation of interneuronal synapses.

The correlation of specialized terminal structure with the effects on SDHACU-dependent synthesis is suggestive of cause and effect, but it is not known if the localization of SDHACU in vivo to the neuronal terminal also occurs in cultured neurons. SDHACU-dependent synthesis in somas was tested in culture conditions which minimized process and thus terminal formation (plain glass substrate) or which limit the formation of stereotypic synaptic structures (late embryonic CG neurons). Both preparations showed significant SDHACU-dependent ACh synthesis, and late embryonic CG neuron cultures showed an enhanced synthetic response to depletion. These data imply that in culture, retrograde trophic influences of the neuronal target tissue include effects upon terminal morphology, but may not be restricted to terminals in regard to transmitter metabolism.
Support: U.S. Army Research Office; NS55402 (RCDA to JBT).

307.11 ACTIVE NEUROMUSCULAR INTERACTION IN CELL CULTURE UNDER SEM Jeremy B. Tuttle, Physiology Section, The Univ. of CT,Storrs, CT Present address: Dept. of Physiology, Univ. of Virginia School of Medicine, Charlottesville, Va. 22908

Embryonic ciliary ganglionic neurons are easily adapted to cell culture. Survival of the neurons is supported by soluble growth factor(s), a killed-cell substrate with high-K+ medium, or co-culture with a muscle target tissue. The neurons rapidly form transmitting neuromuscular junctions (NMJ's) with myotubes, and form interneuronal synapses in vitro, but not in the embryo. As part of a study of the physiological consequences of target interaction, ciliary ganglion neurons were co-cultured with several types of striated muscle target tissue, and examined under scanning electron microscopy. The myotube targets included those with which the ganglionic neurons form lasting active NMJ's and those that are innervated only transiently.

Regardless of the target muscle type, in the early stages of co-culture (3-5 days in vitro) evidence of active interaction between the innervating neurons and the target was prominent. Neuronal processes extending along the myotube surface often encountered areas with short ($\sim$1 μm) finger-like extensions from the muscle surface. In some cases, these occurred at fairly regular intervals along a neurite, crossed and joined around the neurite, and gave the appearance of "cinching" or "tying" the neurite to the target cell surface. In other cases, the muscle extensions formed the edges of a longitudinal depression into which the neurite disappeared. Finally, apparent fusion of these extensions caused the neurites to travel underneath an outer flap of myotube membrane. This form of active target interaction was not limited to the muscle target. Neurites passing over the surface of other neuronal cell bodies were also "cinched" to the surface by similar finger-like extensions. In addition, this interaction was not limited to neuronal processes, as the myotube often surrounded a neuronal soma on its surface with an area of extensions.

The details of neuro-muscular interaction are thought to be complex. Appropriate target recognition and the exchange of macromolecules may both depend upon an active response by neuronal and muscle elements. While the morphological signs of such an interaction in culture may represent an artifact of the in vitro environment, they emphasize the possibility that similar events may occur in vivo and may reflect a fundemental aspect of the neuron-target relationship.
Support by U.S. Army Research Office and NS 55402 (RCDA).

307.12 INTERACTION OF GANGLIOSIDE AND NGF ON PC12 CELLS. S.G. Matta*, G. Yorke* and F.J. Roisen. Dept. of Anatomy, UMD-Rutgers Medical School, Piscataway, NJ 08854.

Murine Neuro-2a neuroblastoma and chick sensory ganglia (DRG) differentiate in response to bovine brain gangliosides. Gangliosides potentiate nerve growth factor (NGF)-induced neuritogenesis of NGF-dependent DRG and NGF-responsive PC12 pheochromocytoma. Antibodies directed against GM1 inhibit NGF-induced DRG differentiation (Schwartz and Spirman, PNAS 79: 6080, 1982). Since gangliosides may serve as modulators in the regulation of NGF-mediated interactions, we examined the effects of GM1, GD1a and GT1b (200 ug/ml, Fidia Res. Labs), alone or in combination with NGF (20 ug/ml) on PC12. Cells were plated in RPMI-1640 + 10% HS + 5% FBS supplemented with test agents for 5 days and the length and number of neurites were analyzed with computer-aided morphometry. NGF-induced sprouting was potentiated only by GM1, which produced a two-fold increase in both process length and number, while the NGF-independent Neuro-2a increased in response to GM1, GD1a and GT1b. In addition to NGF's ability to stimulate neuritogenesis, it independently increases polyamine biosynthesis in PC12, as demonstrated by NGF's induction of ornithine decarboxylase (ODC), the rate-limiting enzyme in this pathway (MacDonnell et al., PNAS 74: 4681, 1977). Accordingly, we determined the effect of the three species on ODC activity. PC12 cells were grown to confluency and incubated for 5 hr in serum-depleted medium containing an individual ganglioside, in the presence or absence of NGF. In contrast to our morphologic results, each ganglioside enhanced ODC levels independently and in concert with NGF. To examine the temporal sequence of ganglioside-NGF interaction, cells were incubated for 24 hr in medium containing GM1 and washed prior to 6 hr NGF exposure. This "priming" induced ODC levels to those obtained with NGF and GM1 simultaneously. This study demonstrates that individual gangliosides affect NGF-induced responses with different degrees of sensitivity depending on the cell model and shows that ganglioside-induced sprouting occurs in the absence of NGF. It also suggests that gangliosides enhance neuritogenesis by an NGF-independent, membrane mediated mechanism, which may have a general role in the regulation of trophic interactions. To examine this possibility, studies utilizing other trophic factors are in progress. NIH grants NS11299 & NS11605.

307.13 SURVIVAL AND PROLIFERATION OF CULTURED CHROMAFFIN CELLS: EFFECTS OF NERVE GROWTH FACTOR (NGF) AND DEXAMETHASONE. L.E. Lillien and P. Claude, Wisconsin Regional Primate Research Center, Neurosciences Training Program and Developmental Biology Training Program, University of Wisconsin-Madison, Wisconsin 53706.

Cultured rat adrenal chromaffin cells respond to NGF by expressing many characteristics of sympathetic neurons (Doupe et al., 1982). Glucocorticoid hormones, including dexamethasome (dex), antagonize this response (Unsicker et al., 1978), apparently by delaying or modulating the NGF response (Lillien and Claude, 1983). In addition, growth in NGF and dex, alone or in combination, results in increased cell numbers compared to control cultures. For NGF this enhancement in cell number (approx. 40-50%) is apparent at 1 and 2 but not consistently at 4 weeks in culture. For NGF plus dex, the enhancement (approx. 40-80%) is apparent throughout a 4 week culture period; enhancement of cell number in dex alone (approx. 40-80%) occurs between 2 and 4 weeks in culture. This effect on cell number could be due to increased cell survival or increased cell division. To determine the extent of cell division under different growth conditions, cultures were exposed to tritiated thymidine (5 uCi/ml) for 24 hr. periods following different times in culture. Whole-mount autoradiograms were prepared, and labeled chromaffin cells were counted. The number of labeled cells in NGF was 3 to 4 times higher than in control conditions at 1 week in vitro and declined to control levels by 4 weeks, which correlates with their becoming more neuronal. Dex reduced this effect of NGF by 40 to 60%. The level of cell division in dex alone was comparable to that in control cultures at all time points. If cells were grown for one week in control medium, and then switched for 2 days to NGF, 2 to 3 times as many cells were labeled in the switched cultures as in control cultures. NGF thus appears to have a mitogenic effect in these cultures. The larger number of chromaffin cells seen in NGF-treated cultures during the first two weeks in vitro can be attributed at least in part to an increase in cell division, while enhanced cell number in dex appears to be due more to enhanced cell survival than to cell division. As in the case of neuritic outgrowth in response to NGF, dex antagonizes the enhancement of cell division induced by NGF.

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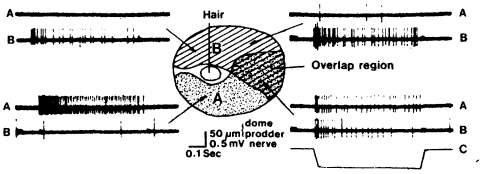
307.14 INTEGRITY OF SENSORY DOMAINS WITHIN SINGLE TOUCH DOMES OF RATS. J. Diamond, G.M. Yasargil*@, L. Macintyre*, R. Doucette. Dept. of Neurosciences, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5

The touch dome of rat hairy skin contains a basal layer of 25-150 Merkel cells (Nurse & Diamond, 1983, Neuroscience 11: 509) supplied by 1-4 myelinated axons. We are using 16 µm diameter prodders to map the mechanosensitivity across single domes; in ones innervated by more than 1 axon we find often that each supplies a functionally discrete territory within the dome, with a variable overlap. The figure shows responses recorded simultaneously in the 2 axons (A & B), innervating this typical shared dome, to a standard stimulus applied at each of 4 locations (C, shown only for 1 location). The borders of domains A and B were constructed from many such tests.



Dome axons, like other "touch" nerves of adult mammals, fail to sprout into adjacent denervated skin, although after crush they regenerate into it (Jackson & Diamond, J. Comp. Neurol., in press). Will a remaining nerve sprout within a partially denervated dome? First we identify single domes shared between 2 axons, one in each of 2 adjacent dorsal cutaneous nerves (DCNs), and map the 2 domains. One of the 2 DCNs is then crushed. To date, no evidence of functional sprouting by the remaining axon has emerged; indeed, the dome's Merkel cells, visualized by their quinacrine fluorescence, were reduced in number (cf. Nurse et al., 1983, Neuroscience 11: 521), but largely within the denervated (insensitive) domain. When the crushed axon regenerates to the dome it restores both mechanosensitivity and Merkel cells (cf. Nurse et al., Neuroscience, in press) to its former domain. We are now examining whether one, or both, axon(s), regenerating to a totally denervated dome, respect former domain borders, and also the EM appearances of Merkel cell-neurite complexes in such potentially competitive situations.

(Supported by MRC Canada)

@ Visiting Scientist from Physiol. Inst. Univ. Zurich

307.15 REGIONAL DIFFERENCES IN THE SENSORY NERVE DEPENDENCE OF MERKEL CELL DEVELOPMENT IN RAT SKIN. L. Mills*, C.A. Nurse, J. Diamond. Dept. of Neurosciences, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5

In mammalian skin clusters of epidermal Merkel cell-neurite complexes function as slowly adapting touch receptors. Whether these Merkel cells are trophically dependent on an intact sensory nerve supply has been a point of controversy. Recent quantitative studies, based on the ability of Merkel cells to accumulate the fluorescent dye quinacrine, indicate that denervation of the touch domes of the dorsal trunk of the rat (hairy) skin between 7 and 60 days of age causes a rapid and persistent loss of ca. 60% of their quinacrine fluorescent (Merkel) cells (QFC) (Nurse et al., 1984, Neuroscience 11: 521-533). We now find that after denervation at 1-3 d of age, about 62% of domes are virtually devoid of QFC by 60 d (mean 1.4 ± 0.5 SEM/dome, n=70); in 1 d controls the mean value was 28 ± 1.7 SEM (n=189), and in 60 d controls, 91 ± 3.9 (n=66). However an entirely different result was obtained for Merkel cells in glabrous skin of the rat footpad. The sciatic or both the sciatic and saphenous nerves of the right hindlimb were cut and ligated; the contralateral unoperated limb served as a control. The extent of the denervation was measured behaviourally (and in some cases, electrophysiologically) 14-21 days later, and approximately 18 hr post quinacrine injection. The smallest (most posterior-lateral) footpad was removed from both feet and its QFC population counted after viewing the separated epidermis in the fluorescence microscope (Nurse et al., 1983, Cell Tiss. Res. 228: 511-524). Footpads denervated at birth - 2 days of age, when the average number of QFC (± SEM) is 112 ± 7 (n=20), continued to acquire new Merkel cells (as do controls) so that between the 2nd and 3rd post-operative week the number of QFC per pad had increased progressively by 6-17 fold; though the denervated footpad was significantly smaller than control, the average density of QFC was always comparable between operated and control pads. In contrast, on the dorsal surface of the lower leg, touch domes that were served by the same transected nerves had a drastic reduction in QFC (by more than 75%) over the same period, and appeared to behave similarly to their counterparts in dorsal trunk skin. Thus the development of Merkel cells in the glabrous skin of the rat hindpaw appears to be largely nerve-independent in contrast to those of the touch domes of hairy skin.

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307.16 INTRACEREBRAL GRAFTING OF NEURONAL CELL SUSPENSIONS: FACTORS AFFECTING SURVIVAL AND GROWTH. A. Björklund and F.H. Gage. Dept. of Histology, Univ. of Lund, Lund, Sweden.

Recent evidence suggests that denervation of the hippocampal formation by fimbria-fornix (FF) lesion results in the release of neurotrophic factors with cell survival and axonal growth-promoting properties (Gage at. al., Nature, 308:637, 1984). The trophic effects of denervation on cholinergic cell suspension survival, growth and transplant volume were assessed in two experiments. Rats were injected with cell suspensions of the fetal septal diagonal band region into the hippocampal formation simultaneously with or without FF transsection. Four to six months later, one group of transplanted animals was injected with diisopropyl-fluorophosphate (DFP) and then, after 4 hours, was sacrificed and the brains were sectioned and stained for acetylcholinesterase (AChE). A second group of transplanted animals was analyzed for choline acetyltransferase (ChAT) activity in the hippocampal formation. The transplant volume of the rats with FF transsection was greater than twice the volume in the animals without FF transsection. In addition, the number of AChE-positive cells in the transplant was also twice as great in the denervated animals than in the non-denervated animals. However, the number of AChE-positive cells/mm^3 did not differ between the two groups, suggesting that the trophic effect of the denervation was not specific for the cholinergic neurons. The ChAT activity of the animals that received a FF lesion simultaneously with transplantation was greater than twice that of the rats which received transplantation but no simultaneous FF lesion. This latter group received FF lesions 7 days before sacrifice in order to reveal the ChAT activity derived exclusively from the transplants. These results strongly support the contention that neurotrophic factors are released as a result of denervation that can support cell survival and growth; however, these factors do not appear to be specific for one type of neuron, but rather have their trophic effect(s) on many or all neurons.

307.17 MATURATION AND SURVIVAL OF DORSAL ROOT GANGLION TRANSPLANTS TO ADULT SPINAL CORD. M.A. Sharkey, R. Lund, R. Dom*. Anatomy Department, Medical University of South Carolina, Charleston, SC 29425.

In a series of anesthetized adult Long Evans rats the dorsal funiculi of rostral cervical spinal cord segments were excised. The resultant cavity was filled with an embryonic day 14 spinal cord segment with attached dorsal root ganglia (DRG), to determine the viability of neural tissue transplanted into an injured spinal cord. Results show that DRG's matured and survived without contacting a peripheral trophic source for nerve growth factor (NGF). One to two months after transplantation the animals were anesthetized, the spinal cord grafts visualized and the spinal cord portion of the graft injected with horseradish peroxidase (HRP:30% solution). Twenty-four hours later the animals were perfused with a phosphate buffered solution containing 0.5% glutaraldehyde followed by a 2% glutaraldehyde phosphate buffered solution. Following fixation the neural tissue was frozen sectioned at 40μm, and alternate sections processed with either diaminobenzidine tetrahydrochloride and counterstained with cresyl violet or subjected to the Holmes fiber stain technique. In two animals the injections were restricted to the spinal portion of the implant.

Results show that the spinal cord grafts were well-integrated with the host, but the DRG remained attached only via the dorsal root to the spinal graft and did not integrate with the host parenchyma. None of the DRG's had processes extending to the periphery; all appeared to be subarachnoid in location. The HRP labeled transplants showed retrogradely labelled cells in the ganglion. Thus, there was no peripheral target tissue from which NGF could be retrogradely transplanted, yet survival and maturation of dorsal root ganglion neurons occurred in this in vivo environment.

307.18 CONTROL OF MUSCLE MEMBRANE STABILITY BY ALPHA-MOTONEURONS. L. Eldridge* and W.F.H.M. Mommaerts. Department of Physiology, University of California at Los Angeles, CA 90024.

The mechanisms by which the spinal alpha-motoneurons maintain electrical stability of the membranes of their target muscles were studied in adult female cats. Membrane instability was assessed by monitoring the occurrence of positive sharp waves, irritability, and fibrillation potentials upon insertion of needle electrodes into the bellies of the medial gastrocnemius and tibialis anterior muscles. In some cases, fibrillation was also checked by direct visual observation of muscles surgically exposed with the cat unanesthetized. Cats underwent one of the following surgeries involving the lumbosacral innervation L5 through S3 serving the lower hindlimb: denervation by ventral root or sciatic section (D), spinal isolation (SI), cord transection (CT), or bilateral dorsal rhizectomy (DR). All cats except DR developed positive sharp waves, irritability, and fibrillation potentials, generally beginning 7 to 9 days after surgery. These signs of membrane instability had disappeared by 4 weeks after surgery in the CT muscles and by 7 to 8 weeks in the SI muscles. The D muscles continued to fibrillate for many months, until they degenerated. The variation in time course of the fibrillation across the treatment groups, as well as among the individual cats in the SI group, suggests that trophic factors are at least partially controlling the changes in membrane stability. First, the difference between the SI and D groups after 8 weeks can not be explained by assuming that SI muscles were more active than D muscles, since the SI fibers were in total flaccid paralysis, less active than the fibrillating denervated ones at all time points after 8 weeks. Second, although the CT cats by 6 days after surgery had recovered from spinal shock, showed hyperactive relfexes, and demonstrated considerable motor unit activity even while their reflexes were not being deliberately stimulated, they did not show maximal signs of membrane instability until 3 weeks after surgery. Third, the onset of fibrillation in the SI cats ranged from 5 to 17 days after surgery. The latency of onset varied directly with length of the legs, with the cat having the longest legs showing 17 day delay.

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307.19 SOLUBLE PROTEINS OF RAT MUSCLE: EFFECT OF DENERVATION AND NERVE EXTRACT. E.A. Heinicke* and H.L. Davis. Departments of Anatomy and Biochemistry, The University of Western Ontario, London, Ontario N6A 5C1 Canada

Denervation of a skeletal muscle results in atrophy of the muscle and loss of total protein. These changes result from both disuse and loss of neurotrophic substances. Aqueous extracts of rats' or sheep's sciatic nerves have been shown to reduce some of the protein loss when injected into rats' extensor digitorum longus (EDL) muscles which have been denervated seven days.

This study investigates the effect of denervation on individual proteins of the sarcoplasm of EDL muscles of the rat and assesses the effect of injected sheep nerve extract in offsetting these changes. Right EDL muscles were denervated by removal of sciatic nerve from the thigh. Denervated and contralateral normal muscles were dissected out after seven days and homogenized in dilute phosphate-buffered saline. Insoluble protein was removed by centrifugation and the amount of protein in the supernatant was determined biochemically. The extracted proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, stained with Coomassie Blue and scanned spectrophotometrically. The relative amount of protein in each band was determined from the area under its absorbance peak and expressed as a fraction of the total protein on the gel. Molecular weights were estimated by comparison with known standards. The relative amounts of protein in corresponding bands from normal and denervated muscles were compared. The total soluble protein was significantly lower in the denervated muscles than in normal contralateral controls. A number of electrophoretically separated proteins were proportionately increased in the denervated muscles and some were relatively diminished. Daily intramuscular injections of extract of sheep's sciatic nerves ameliorated some of the denervation-induced decrease in the total soluble protein. The effect of the neurotrophic factor on the relative distribution of soluble proteins was also examined electrophoretically.

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307.20 DENERVATION OF STERNOMASTOID MUSCLE IN TREMBLER DYSMYELINATING MUTANT AS COMPARED TO CONTROL MOUSE ; H.L. KOENIG[1], N.A. DOTHI[2], M. VIGNY[3].
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3 ENS Paris, France.

Trembler mutation is characterized by a segmental dysmyelination of peripheral nerves. Motor innervation, ACh-receptors and AChE activity are modified in fast and slow muscles. We investigated the possible differences in the effects of denervation in sternomastoid muscle of Trembler and Control mice. Several differences were observed in Trembler denervated muscle 1) the total AChE activity in the whole muscle decrease more rapidly in Trembler. 2) the relative proportion of asymmetric forms (A12 + A8) declined more rapidly in the mutant, whereas the increase of globular forms (G1 + G2) was similar. In both mice G4 AChE disappeared completely after 1-2 weeks. Even after 4 weeks of denervation, collagen-tailed forms were still present in both nerve free and endplate regions in Trembler and control. 3) in the contro-lateral sternomastoid, the slight decrease of A12 + A8 forms was also more rapid in Trembler. 4) small patches of extrajunctional ACh-receptors were observed at distances of 0,3 to 3mm from the denervated endplates, in Trembler and control. In Trembler, the appearance of the patches was delayed by 1 week, they appeared only after 2 weeks of denervation. 5) occasionally, the ACh-receptor patches were colocalized with identical immunostained patches of AChE in Trembler and control. The denervated endplates exhibited always simultaneous AChE and ACh-receptor staining.

307.21 REGULATION OF MUSCLE SIZE BY ACTIVITY AND NON-ACTIVITY-RELATED FACTORS S. A. Spector. Dept. of Kinesiology, UCLA, Los Angeles, Ca. 90024.

In view of fluctuations in muscle size induced by modified activity levels in such pertubations as high resistance weight training or joint immobilization, it is thought that the size of skeletal muscle depends to a large extent on the amount or pattern of neuromuscular activity. However, it can not be concluded a priori that non-activity-related factors, thought to regulate some electrophysiological properties of muscle, does not influence muscle size.

To test the possibility of a non-activity-related effect on size of muscle, soleus muscle weight (MW) and mean fiber cross-sectional area (F-CSA) were assessed bilaterally in adult female rats (180-200g) which underwent hindlimb denervation contralateral to chemically induced neuromuscular paralysis. Denervation (D_2) was produced by sciatic nerve excision in the mid-thigh region; paralysis (T_2) was induced with tetrodotoxin applied chronically to sciatic nerve via a mini-osmotic pump whose contents were led to a Silastic nerve cuff. Bilateral determinations were made on a second group of denervated (D_1) and sham-operated (C_1) rats. After 2 or 4 weeks of treatment, each muscle was excised and weighed, and the cross-sectional areas of between 100-150 fibers were digitized.

The results (X ± SEM) listed in the Table show that, for both MW and F-CSA assessed after 2 or 4 weeks, decreases in these values with denervation are significantly attenuated ($P<0.05$) for T_2. The extent of differences between D_2 and T_2 for both MW and F-CSA for either period of treatment is between 20% and 30%. These results suggest a substantial influence of neuromuscular activity in controlling the size of muscle. In addition, however, the present findings necessitate the postulation of a non-activity-related factor which presumably is released by the motoneuron and which controls, to a lesser but significant degree, the size of the muscle and its fibers.

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	2 weeks			4 weeks		
	MW	F-CSA	n	MW	F-CSA	n
C_1	101 ± 4	2337 ± 132	7	120 ± 4	2457 ± 122	8
D_1	51 ± 2	1023 ± 96	7	46 ± 2	741 ± 91	8
D_2	52 ± 3	965 ± 58	8	43 ± 2	656 ± 80	6
T_2	67 ± 4	1154 ± 73	8	56 ± 4	864 ± 107	6

307.22 TROPHIC SUPPORT BY THE OTOCYST OF CHICK COCHLEO-VESTIBULAR NEURONS IN ORGAN CULTURE: ROLE OF CELL DIVISION. S.H.Hauger and D.K. Morest. U. Conn. Health Center, Farmington, CT 06032

We are studying interactions between the neurons of the cochleo-vestibular ganglion (CVG) and their peripheral synaptic targets, auditory and vestibular hair cells derived from the embryonic otocyst. When explanted at stage 21-23 (E3½-4) and maintained in organ culture for 14 d, the CVG contains 416 ± 257 (SD) neurons (range 267-773). However, if the otocyst is included in the explant, the number of CVG neurons rises to 2843 ± 1132 (range 1579-4925) (Ard, Morest & Hauger, submitted). This difference was attributed to trophic support of the CVG by the otocyst; i.e., neurons survived and differentiated because of the presence of the otocyst.

In the present study, an alternative interpretation was tested: the additional neurons occurring with the otocyst present are generated by cell division. This was tested by maintaining organ cultures of combined otocyst and CVG at stage 18½-24 for 14 d in the continuous presence of ^{3}H-thymidine (0.25, 0.5 or 1.0 µCi/ml). Three doses were used as a control for possible toxic effects of tritium; no consistent dose-related differences were seen. Other culture conditions were as before (Ard & Morest, '81, Soc. Neurosci. Abst. #249.17). The cultures were fixed in Bouin's, embedded in plastic, serially sectioned at 5 µm, and processed for autoradiography. Neurons were identified after staining with toluidine blue, cresyl violet or thionin.

The only predominantly unlabeled cells were the CVG neurons -- less than 0.1% of these cells were labeled above background. Many of the other kinds of cells had clearly labeled nuclei, indicating that they had divided in vitro. These included hair cells, supporting cells and other epithelial cells, cartilage, connective tissue and peri-neuronal satellite cells.

To find out if neuroblasts did divide in culture but were killed by tritium, the neurons in each culture were counted. Indeed, cultures from stage 18½-22 embryos had few neurons (less than 800). However, for cultures from stage 23-24 embryos, the numbers of neurons were in the range reported by Ard et al. (see above).

We conclude that all neurons surviving 14 d in vitro must have been present and postmitotic at the time of explantation. Neuroblasts, if present, either failed to divide or failed to produce viable or recognizable progeny. Thus the possibility that the trophic interaction between the otocyst and the CVG neurons is mediated by cell division seems unlikely.

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307.23 NERVE GROWTH FACTOR REGULATES ACETYLCHOLINESTERASE IN SYMPATHETIC GANGLIA. L.M. Ibsen* B.A. Hay*and C.G. Reiness. Department of Biology, Pomona College, Claremont, CA 91711.

We have investigated whether the forms of acetylcholinesterase (AChE) in rat sympathetic ganglia are regulated by nerve growth factor (NGF). Velocity sedimentation analysis of extracts of superior cervical ganglia (SCG) from male Sprague-Dawley rats on linear sucrose gradients revealed AChE forms sedimenting at 16S, 10S and a doublet at 4-6S (cf Gisiger et al., J. Neurochem. 30, 501 (1978)). Because of seasonal variations in levels, we report only data from studies conducted in summer. We estimated the relative proportion of the forms graphically as 12.4±2.1% 16S AChE, (mean±S.D., n=13), 41.3±4.2% 10S and 46.3±4.4% 4-6S. Approximately 85% of total cholinesterase and of each form was AChE, as judged by sensitivity to iso-OMPA and BW 284c51.

Depriving the ganglion of NGF by axotomy of both the external and internal carotid branches caused a 40-50%decrease in total AChE/ganglion after 6-8d. There was a selective, significant decrease in the proportion of the 16S form in the remaining AChE in the axotomized ganglion compared with the control ganglion from the same animal (4.5±0.9% vs 12.1 ±2.3%, n=4; $p<0.02$). There was a small, nonsignificant increase in 10S AChE (52.6±7.9% vs 45.6±3.2%) and no change in 4-6S AChE (42.9±7.2% vs 42.3±3.4%) in axotomized ganglia. Thus axotomy selectively reduces the relative proportion of 16S AChE by >60%.

Immunization of rats with NGF also caused selective reduction of 16S AChE levels. Immunization caused reductions in total protein (21%), total AChE (19%), and tyrosine hydroxylase (51%) levels in SCG. The remaining AChE showed a significant reduction in the proportion of the 16S form (8.9±3.1%, n=7 vs 12.4±2.1%; $p<0.01$); no significant differences in 10S (44.4±6.8%)or 4-6S AChE (46.6±6.6%) were seen.

Daily intravenous injections of exogenous NGF for 6-8d (2.5 mg/kg body wt/d) appeared to increase 16S AChE levels in both axotomized (5.6±0.9% vs. 4.5±0.9%, n=4) and control ganglia (16.0±2.2% vs 12.1±2.3%, n=4), but the differences were not statistically significant ($p=0.06$).

Therefore, treatments which probably reduce NGF levels in SCG cause a selective loss of 16S AChE, while addition of exogenous NGF may increase 16S AChE levels. These results indicate that levels of 16S AChE in sympathetic ganglia are regulated by levels of NGF, as are many other properties of ganglionic synapses (Purves and Lichtman, Physiol. Rev., 58 821 (1978).

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307.24 CONTROL OF CEREBELLAR CELL PROLIFERATION IN STAGGERER, LURCHER AND HYPOTHYROID MUTANT MICE. Anne Messer and Kathleen Hatch.* Center for Labs. and Research, N.Y. State Dept. of Health, Albany, N.Y. 12201.

The numbers and types of cells which are destined to interact in the mature cerebellum seem to be controlled by a variety of developmental mechanisms. Questions of which processes are intrinsically controlled by the genes, and which are controlled indirectly either by signals from other cell types or via systemic hormones can be powerfully investigated using mutations which interfere with the normal development of the cerebellum, and/or by hormonal manipulations which alter developmental sequences. Here we address the mechanism of action controlling proliferation in the cerebellar external granule layer (EGL) by comparing hypothyroidism in mice, using the hypothyroid (hyt/hyt) mutant, to the staggerer (sg/sg) and Lurcher (Lc/+) cerebellar mouse mutants. The 3 mutants differ in the time-course and severity of their Purkinje cell defects, and show concomitant abnormalities in their patterns of EGL proliferation.

In these experiments, levels of the enzyme thymidine kinase (TK) are used to assess DNA proliferation biochemically, with histological studies and autoradiography after 3H-thymidine uptake used to correlate the enzymology with in vivo observations. Previous work has shown that induction of TK activity in developing sg/sg mouse cerebellum is greatly reduced during the normal peak of proliferation (days 6-8). Lc/+ shows no such early reduction, while hyt/hyt mice to date have shown greater variability than controls.

Effects of the three mutations on the cessation of proliferation are even more striking. In all cases there is an extension of the proliferative period, as if the EGL cells fail to receive a signal to stop dividing at the proper time. Sg/sg mice show higher than normal levels of TK at days 14, 18 and 22; hyt/hyt at days 14 and 18, and Lc/+ on days 18 and 22. This roughly reflects the constant, early and later effects of the mutations on Purkinje cells respectively, and can also be correlated with an extended time for observation of the EGL histologically and with cell types shown to take up 3H-thymidine in vivo. Implications for the hypothesis that Purkinje cell abnormalities in all 3 mutants lead to abberent signalling of the onset and cessation of proliferation in the EGL will be discussed.

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308.1 HORMONAL REGULATION OF TRANSLATABLE mRNAs OF SPECIFIC GLIAL PROTEINS IN C6 CELLS. S. Kumar*, D. P. Weingarten*, J. Callahan*, K. Sachar* and J. de Vellis. Lab. of Biomed. and Environmental Sciences, Mental Retard. Res. Center, UCLA School of Medicine, Los Angeles, CA 90024.

The regulation of translatable mRNAs of three glial enzymes, namely, glycerolphosphate dehydrogenase (GPDH) and glutamine synthetase (GS) in response to hydrocortisone (HC), and lactate dehydrogenase (LDH) in response to norepinephrine (NE), is described in C6 cells. Specific mRNAs were obtained by affinity purification of GPDH, GS or LDH polysomes on IgG coupled Seph 4B. Using cell-free translations of GPDH-, GS-, or LDH-polysome enriched poly(A)$^+$ RNA, we report a dramatic increase in translatable mRNAs of these enzymes in the presence of hormone as compared to uninduced cells. The hormonally induced appearance of these translatable polysomal poly(A)$^+$ RNA are sensitive to actinomycin D, an inhibitor of RNA synthesis indicating the transcriptional regulation of these glial enzymes. The NE-modulated transcription of LDH or the HC-mediated transcription of GPDH are dependent upon the synthesis of protein(s) as evidenced by cycloheximide sensitivity. The HC-mediated induction of GS specific mRNA, however, is not dependent upon protein synthesis. While a short-chain fatty acid, sodium butyrate (NaB), abolishes all HC-inducible GPDH specific mRNA, the HC-mediated GS specific mRNA is enhanced when in vitro cell-free translation products were analyzed by immuno- precipitation. The magnitude of NaB inhibition of HC inducible GPDH or increase of GS-induction paralleled the GPDH enzyme inhibition and GS enzyme induction of HC in the presence of NaB in these cells. Millimolar concentrations of NaB and other short-chain fatty acids in mammalian cell culture are known to produce a reversible hyperacetylation of core histone proteins which play some important role in the control of chromatin transcriptional activity. We observed an increased hyperacetylation of histone proteins with increasing length of fatty acid chain. A same order of effectiveness of inhibition of the HC-mediated GPDH followed with these fatty acids along with the same order of effectiveness in induction of GS.

This work was supported by DOE Contract DE-AM03-76-SF00012, and NICDH HD-06576-11.

308.2 INDUCTION BY A TUMOR PROMOTER OF RNA-DEPENDENT DNA POLYMERASE IN HUMAN BRAIN TUMOR CELLS GROWN IN CULTURE. R.P. Uteg*, M. Javid, H.I.W. Kao* and H. Kubinski. Division of Neurosurgery, University of Wisconsin School of Medicine, Madison, WI 53706.

Several authors reported on the phenomenon of the induction by dexamethasone and by various analogs of purines and pyrimidines of virus-like particles in cell lines derived from tumors induced in experimental animals. The presence of such particles can be demonstrated by a variety of techniques including determination of the DNA polymerase in the medium (the so-called reverse transcriptase). In the present study human brain tumors obtained during surgery were grown in vitro. Such cultures were then challenged with a variety of iodinated and brominated analogs of purines and pyrimidines and with dexamethasone. DNA synthesis was tested using calf thymus DNA, a synthetic polymer poly(A):oligo(dT), or culture media and cell extracts without any external template. We were unsuccessful in our attempts to induce viruses in more than 60 of such tumor-derived cultures. Next, we tested the effects of tumor promoters. As a typical tumor promoter we used 4β-phorbol 12β-myristate 13α-acetate (TPA). The cells were exposed to this compound at micromolar concentrations for periods up to 48 hrs and at 37^{0}C. DNA synthesis was observed in the cell culture media and in the post-mitochondrial supernatants from the disrupted cells using either of the two nucleic acid templates or the internal template. The degree of this enzymatic activity was related to the amounts of the promoting chemical applied. No induction was seen with dimethyl sulfoxide (DMSO) used as a solvent for TPA. Samples of media from the induced cultures and the post-mitochondrial supernatants were centrifuged until equilibrium in sucrose gradients. The DNA-synthesizing activity was recovered from the gradients at the density close to 1.18 g/ml, characteristic for RNA-containing oncogenic viruses (retroviruses). Further studies are needed to decide whether these virus-like particles are in any way related to other known retroviruses. The relevance of our observations to the etiology of brain tumors in humans and the usefulness of such observations to the therapy of brain tumors remain to be explored. - Supported in part by the Consultation Practice Plan of the University of Wisconsin Medical School.

308.3 TRANSCRIPTIONAL REGULATION IN THE HYPOTHALAMIC-PITUITARY AXIS. M.Blum+, B.S.McEwen†, and J.L.Roberts#. +Dept. of Neurobiology, Rockefeller Univ. and #Center for Reproductive Sciences, Columbia Univ., New York, NY.

The tuberoinfundibular dopaminergic neurons (TIDA) have been demonstrated to inhibit the release of prolactin (PRL) from the anterior pituitary (AP) and pro-opiomelanocortin (POMC) from the intermediate pituitary (IP). It has been previously shown that dopamine (DA) decreases transcription of PRL from primary cultures of AP and POMC from IP cultures suggesting that changes in peptide release are reflected in changes of the transcriptional activity of the gene. Tyrosine hydroxylase (TH) is the rate limiting enzyme in the synthesis of DA and changes in its activity appear to be a good indicator of electrical activity or release of DA. Variations in DA turnover and synthesis have been detected during the rat estrus cycle. After ovariectomy these cyclic changes in the TIDA immediately dissapear. Using a nuclear runoff transcription assay, studies are in progress to test whether changes in TH gene transcription in the arcuate nucleus can be detected during the estrus cycle, reflecting the cyclic changes seen in DA release. Concomitantly we are measuring transcription of AP-PRL and IP-POMC during the estrus cycle and in OVX/steroid replaced paradigms. Nuclei isolated by differential centrifugation are incubated with ^{32}P NTPs, allowing for the RNA nascent chains initiated in vivo to be elongated in vitro. Specific RNA transcripts are identified by hybridization to TH, POMC, and PRL cDNAs. The number of cpm incorporated into a specific RNA transcript reflects the number of RNA polymerases on the gene at the time of sacrifice. Levels of incorporation of radioactive RNA precursors into RNA from nuclei isolated from the arcuate nucleus was sufficient to allow for the detection of TH gene transcription. Preliminary results gave a value of 30ppm in arcuate nuclei that had been taken from males, which is sufficient to detect both increases and decreases in transcriptional activity resulting from a changing hormonal environment. When transcription of POMC in IP primary cultures was measured to distinguish between direct and indirect effects of estrogen, it was found that estrogen decreases POMC gene transcription by approximately 40%. (This work was supported by grants NS07078 to MB, NS07080 to BSM, and AM27484 JLR).

308.4 TRANSCRIPTION OF THE RAT BETA LH GENE IS STIMULATED BY GNRH. J.A. Jonassen* and J.L. Roberts. International Institute for the Study of Human Reproduction, Columbia University College of Physicians and Surgeons, New York, NY 10032.

Pituitary luteinizing hormone (LH) secretion is stimulated by gonadotropin releasing hormone (GnRH) and is also regulated by positive and negative feedback effects of estradiol (E) on the hypothalamo/hypophyseal axis. Molecular mechanisms by which GnRH and E modulate LH secretion are complex and not clearly understood. The present studies were performed to determine if effects of E or GnRH on LH secretion were associated with changes in transcription of the beta (β) subunit of the LH gene. To this end, the rat βLH gene was isolated from a lambda Charon 4a phage rat genomic library and characterized by restriction enzyme mapping. A 2.1 kb DNA fragment, containing the entire βLH gene but no middle repetitive DNA sequences, was used in these studies. Adult rat pituitaries were enzymatically dispersed, primary pituicyte cultures established for 4 days and either 10^{-8} M E was added for an additional 48 hr or 5×10^{-7} M GnRH was added for 4 hr. Cells were permeabilized with digitonin and elongating nascent hnRNA was labeled with ^{32}P [UTP] and ^{32}P [GTP]. The proportion of βLH RNA transcripts labeled during the elongation reaction was assessed by hybridization of purified, ^{32}P-labeled pituicyte hnRNA to the filter-bound 2.1 kb DNA fragment containing the rat βLH gene. E treatment for 48 hr led to slight, but reproducible decreases in βLH gene transcription, from 75 ppm in control pituicytes to 55 ppm in E-treated cells. In distinct contrast, GnRH treatment for 4 hr markedly enhanced βLH gene transcription 5-20 fold. Thus, GnRH directly stimulates both transcription of the βLH gene and LH secretion in cultured rat pituitary cells. In contrast, absence of a pronounced effect of E on βLH gene transcription may indicate that E modulation of LH secretion is not accompanied by direct transcriptional effects or that it occurs at a site distinct from the gonadotroph. (Supported by NIH 1R23HD18710-01 to JAJ and a Ford Mellon Rockefeller Grant to JLR).

308.5 MOLECULAR BASIS FOR THE DIFFERENCES IN EXPRESSION OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE IN TWO STRAINS OF RAT. M.J. Evinger*, E.E. Baetge, D.H. Park, V.R. Albert, D.J. Reis and T.H. Joh (SPON: B. Judd). Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

The activity of the epinephrine synthesizing enzyme, phenylethanolamine N-methyltransferase (PNMT, E.C. 2.1.1.28) differs in the brain and adrenal medulla in rats of the Buffalo (Buf) and Fischer (F344) strains. Thus, studies by Stolk and associates (Vantini et al., Brain Res., 1984) have demonstrated PNMT activity is 90 and 65% less in hypothalamus and pons medulla, respectively, in Buf as compared with F344 controls. We have sought to establish whether differences in PNMT activity in these strains can be attributed to differences in amount of enzyme protein, and in turn, amount of specific mRNA for PNMT.

Studies were conducted in male Buf and F344 rats weighing 200-250 g. In agreement with others, PNMT activity was lower in pons medulla and adrenal medulla of Buf than F344, 43% and 21%, respectively. PNMT in adrenal homogenates prepared from both strains was immunostained on Western blots. Antibodies to bovine adrenal PNMT recognize a single protein band in both strains with substantially less staining of PNMT protein in Buf rats. To determine whether differences in immunoreactive PNMT were paralleled by quantitative differences in specific mRNAs, PNMT mRNA from adrenal and brain stem of both strains was analyzed using a quantitative dot blot hybridization assay capable of detecting less than 5 pg of the message. Nick-translated PNMT cDNA (Baetge et al., Int. J. Neurochem., 1983) served as the hybridization probe. Poly A^+ RNAs were isolated from brain stem and adrenal medulla of both strains and fixed to nitrocellulose filters. Based on computer assisted image analysis of autoradiograms, PNMT mRNA of adrenal and brain stem of Buffalo rats was proportionately lower than that from rats of the F344 strain.

These studies indicate that strain specific differences of PNMT activity in brain and adrenal of Buf and F344 rats are attributable to differences in amount of enzyme protein, and as such, appear to reflect corresponding differences in the amounts of specific mRNA for the protein. (Supported by NIH Grants NS19002, MH24285 and HL18974.)

308.6 COUPLED CELL-FREE TRANSLATION AND TWO-DIMENSIONAL GEL ANALYSIS OF RAT BRAIN POLYSOMES ISOLATED DURING FUNCTIONAL PHENOBARBITAL TOLERANCE. W.A. Walker, D. Haeckel*, W. Dietz* and M.J. Mycek*. Dept. of Research, Queen's Med. Ctr Honolulu, HI 96808.

The role of protein synthesis in the development of functional barbiturate tolerance has been suggested by Hitzmann and Loh in studies using protein synthesis inhibitors to block tolerance and in studies using in vivo incorporation of labeled amino acids to measure protein synthesis (Eur. J. Pharmacol. 40 163, 1976; Life Sciences 20 35, 1977). We have attempted to monitor brain gene expression in more detail during the development of tolerance to phenobarbital (PB) by using a cell-free translational system coupled with two-dimensional (2-D) gel electrophoresis. According to this method, the polysomal RNA populations of cortex and subcortex were isolated after 24 hr. and 96 hr. of PB exposure and translated in a wheat germ system into (^{35}S-Met)-labeled proteins. The radioactive translation products were separated on 2-D gels using the combination of non-equilibrium pH gradient electrophoresis and SDS-linear gradient polyacrylamide gel electrophoresis. The resolved translation products were visualized by fluorography. Over 600 different mRNA translation products could be detected and quantified.

Translational activity as measured by incorporation of (^{35}S)-Met into trichloroacetic acid precipitable protein was significantly increased in the polysomal mRNA fraction isolated from the subcortex after 24 hr. of PB exposure while mRNA activity in the cortex was unchanged. This activation of mRNA activity correlates in time with the first measurable display of tolerance. 2-D gel analysis showed that the increase in subcortical mRNA activity was due to the selective activation of specific mRNAs and not due to an overall increase in the translational activity of all mRNAs. In particular, (^{35}S)-Met incorporation was significantly increased in a 53,000 MW translation product tentatively identified as tubulin. No significant differences in the mRNA activity of cortex or subcortex was detected after 96 hr of PB treatment, a time point at which tolerance is maximal.

These results indicate that there is a major activation of gene expression that takes place in the subcortex within 24 hr of PB treatment and that only the products of certain genes are activated. Further experiments are planned to identify which, if any, of these gene products are involved in the process of barbiturate tolerance.

308.7 RNA ISOLATION FROM ALZHEIMER'S BRAIN. M.R. Morrison, S. Jamison*, S. Ojeda*, S. Pardue*, and W.S.T. Griffin. Departments of Neurology, Cell Biology, and Physiology, University of Texas Health Science Center at Dallas, Dallas, Texas, 75235.

There are characteristic deficits in various cell populations and neurotransmitters in Alzheimer's disease (SDAT) and overall energy metabolism in cortex may be compromised. However, histological analyses of neuronal RNA content does not show whether there is a decrease in neuronal RNA content over and above that characteristic of the normal aging process. We and others have recently shown that translationally active messenger RNAs, representative of those present in vivo, can be isolated from postmortem human brain (Morrison and Griffin, 1981, Anal. Biochem. 113, 318; Gilbert et al, 1981, J. Neurochem. 36, 976) and RNA microisolation techniques (Morrison et al, 1984, J. Cell. Biochem., Supp. 8B, 103) now allow us to contrast the RNA isolated from affected and unaffected areas in SDAT brains with that isolated from equivalent areas of control brain. We first compared recoveries of total RNA from SDAT and normal cortex when RNA was isolated from micropunches by our standard phenol extraction procedure. We were able to reproducibly isolate 0.8-1 µg total RNA per mg wet weight control cortex. Recovery of total RNA from SDAT cortex was 80-90% that of the controls, showing that there was not a dramatic loss of total RNA in affected cortex. In order to compare specific mRNA levels, ^{32}P-nick translated tubulin and actin recombinant DNA probes were hybridized to slot blots of control and SDAT RNAs. Hybridization levels were similar showing that specific mRNAs are not appreciably degraded in SDAT compared to control cortex. Our results are in contrast to those of Sadjel-Sulkowsa et al (1983, Banbury Report 15, 193) who report low RNA recoveries from control cortex (0.095 µg/mg tissue) and a 50% loss of total RNA from SDAT cortex. The translational activity of RNAs isolated from control and SDAT micropunches using the cesium chloride technique is now being analyzed. More extensive comparisons are also being made between areas in SDAT brain compromised histologically and those which appear normal by histological criteria.

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308.8 CHARACTERIZATION OF ZINC-BINDING LIGANDS IN RAT BRAIN. M. Ebadi, Dept. of Pharmacology, The University of Nebraska College of Medicine, Omaha, NE, 68105.

Proteins that bind zinc may be classified into at least three major groups consisting of a) metalloenzymes, b) metallothioneins, and c) metalloproteins other than metalloenzymes or metallothioneins. By using Sephadex G-75 column chromatography, we have detected three zinc-binding proteins, with molecular weights of 13,000-15,000, 25,000 and 210,000 daltons respectively. The synthesis of the low molecular weight protein, most probably a metallothionein, is stimulated in a dose-dependent fashion (0.02-0.22 µmoles zinc/µl/hr/48 hrs) following intracerebroventricular, but not intraperitoneal, administration of zinc sulfate. Copper is able to stimulate the synthesis of zinc thionein in the brain, but does not bind to it. Cadmium competes with zinc for binding site, but no endogenous cadmium binding ligands have been detected in rat brain. Actinomycin D (1.5 µg/hr for 48 hrs) was able to block the zinc-induced stimulation of zinc thionein. Furthermore, zinc stimulated the incorporation of L-[^{35}S]cysteine into zinc thionein. The incubation of zinc thionein with $^{65}Zn^{2+}$ and chromatographies on Sephadex G-50 and ion exchange columns of DEAE Sephadex A-25 produced peaks which migrated to the same position as the [^{35}S]cysteine. Other studies using chromatofocusing have shown that the binding affinity of zinc for zinc thionein is greatly enhanced by dithiothreitol (Fig. 1). The results of these studies suggest that specific zinc thionein and other non-thionein-like zinc-binding ligands do exist in the brain. (Supported in part by a grant NS-08932.)

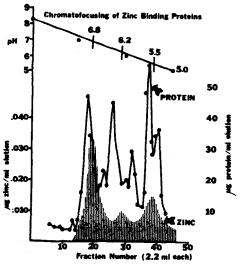

Fig. 1

308.9 PROTEIN-O-CARBOXYLMETHYLTRANSFERASE IS LOCATED IN RAT BRAIN NEURONS. M.L. Billingsley and D.M. Kuhn*. Section on Biochemical Pharmacology, NHLBI, NIH, Bethesda, MD 20205.

The distribution of the enzyme protein-O-carboxylmethyltransferase (PCM; E.C. 2.1.1.24) has been investigated in the rat brain using both immunohistochemical and biochemical techniques. The enzyme, which carboxylmethylates aspartic and glutamic acid residues of protein substrates, was localized in neurons, but not other cell types throughout the brain. Coronal slices of rat brain (60-100 μM) were incubated with PCM antisera (1:1000), followed by a biotinylated goat antirabbit IgG. Immune complexes were visualized after incubation with an avidin-peroxidase complex and color development with diaminobenzidine. The highest PCM immunoreactivity was detected throughout the cortex, followed by the hippocampus, the corpus striatum, the thalamus, and the amygdala. PCM immunoreactive cells were also detected throughout other brain regions. PCM immunoreactive pyramidal and granule cells were found throughout the hippocampus (CA1-CA4). Pyramidal cells and their ascending axons were labelled in the cerebral cortex. Neurons in the corpus striatum were uniformly labelled, suggesting that PCM was not localized to any specific neurotransmitter system. The antisera was generated against purified bovine brain PCM and western immunoblot analysis indicated cross reactivity with both rat brain and human erythrocyte forms of PCM. PCM enzyme activity and methyl acceptor protein capacity was examined biochemically, and highest enzyme activities were found in coronal sections of cortex and corpus striatum, or cortex and hippocampus. The lowest enzyme activities were in slices of brainstem and cerebellum, areas with low amounts of immunoreactive PCM. Methyl acceptor protein capacity was highest in slices of cortex and corpus striatum, cortex and hippocampus and was lowest in slices of brainstem and cerebellum. These results demonstrate that protein-O-carboxylmethyltransferase has an unique neuronal pattern of distribution in the rat brain, suggesting a specific role for carboxylmethylation in these neurons.

308.10 AN ENZYME LINKED IMMUNO-SORBENT ASSAY FOR HUMAN LEUKOCYTE INTERFERON. R.Siddhartham*, W.A.Sheremata and A.Sazant. Neuroimmunology Labs, Dept. Neurol., Univ. of Miami Sch. of Med., Miami, FL 33101

Clinical trials of human leukocyte interferon (Hu-IFNα) in neurological disorders particularly multiple sclerosis have increased in number. We present an accurate, easily reproducable technique for the quantification of Hu-IFNα in body fluids as a necessary corallary of such investigations.

We have successfully developed a solid phase immunoassay, based on the indirect ELISA. Results are standardized against a laboratory reference standard. The procedure is outlined: 1)serial dilution of Hu-IFNα in coating buffer (carbonate-bicarbonate buffer pH 9.4) are carried out in quadruplate wells of polystyrene microtiter plates. Antigen is passively adsorbed onto the solid phase by incubating the plates at 37°C. for 3hrs.; 2)the protein binding sites are then blocked by filling the wells with 1% Bovine serum albumin in diluent (phosphate buffered saline, pH 7.2) for 30min. at 25 C.; 3)200μl of polyclonal sheep anti Hu-IFNα in diluent is pippeted into the wells and the plates incubated at 4°C. for 2hrs. for optimal antigen-antibody binding; 4)200μl of 1:15000 conjugate (peroxidase conjugated IgG fraction rabbit:anti-sheep IgG F(ab')2 fragment specific)in diluent is pippeted into the wells and incubated at 20 C. for 2hrs.; 5)200ul of substrate (40mg/100ml of o-phenylene diamene in citrate-phosphate buffer pH 5.0 with .01% H_2O_2 is added immediately before use). The enzymatic reaction is stopped with 50ul of 5M citric acid and absorbance of the end products determined at 450nm after 30min. at 25°C. Plates are washed with distilled water after each stage.

Our assay is automated and utilizes immunological reagents that are freely available. Large batches of test samples can be assayed and results obtained at the end of the day. The assay provides reliable quantification of Hu-IFNα in cerebrospinal fluid and serum and offers advantages over the time-consuming virus plaque inhibition assays, while offering comparable sensitivity to radio-immunoassays. Detection limits can be as low as 100 IU/l depending on incubation times and antibody dilutions. (Supported by PHS grant NS 17238.)

308.11 ENZYMATIC DEPHOSPHORYLATION OF NEUROFILAMENTS (NFs). M.J.Carden*, R.A.Angeletti*, W.W.Schlaepfer* and V.M-Y. Lee* (SPON:N.K.Gonatas). Divn. of Neuropathology, University of Pennsylvania Med. Sch., Philadelphia, PA 19104.

Mammalian NFs are composed primarily of three polypeptides, the triplet, with apparent mol.wts. on SDS gels of around 200K (NF200), 150K (NF150) and 70K (NF70). NF200, and to a lesser extent NF150, are highly-phosphorylated in freshly-isolated bovine NFs [J.B.C., 257:9902 1982].

We have incubated NFs from bovine spinal cord with alkaline phosphatase from E.Coli. This causes a progressive alteration of gel mobilities for NF200 and NF150 bands. After <1hr. single, sharply-defined polypeptide bands are produced which, on 3-12% polyacrylamide gradient gels (Laemmli type), have apparent mol.wts. 150K and 140K, respectively. The mobility of NF70 remains unaltered. Longer incubation or addition of fresh enzyme does not produce further mobility changes and all effects are abolished by phosphate (an inhibitor of phosphatase).

Modified (de-phosphorylated) forms of NF200 and NF150 sediment completely with filaments, no protein being detectable in the supernatant. Preliminary experiments on the limited proteolytic digestion patterns generated from dephosphorylated NFs, using chymotrypsin or calcium-activated protease from bovine brain, suggest that mofdifications to NF150 and NF200 occur along the portions of these components that are not involved in filament 'backbone' formation.

Freshly-isolated NFs contain kinase activity that causes ^{32}P-incorporation into the triplet when NFs are incubated with $\gamma^{32}P$-ATP. This activity does not re-phosphorylate NF polypeptides after treatment of NFs with phosphatase.

Freshly-isolated or dephosphorylated NF samples have been immunoblotted with >100 monoclonal antibodies (MAs) and reactivity of all NF70-specific MAs (14) is unaffected by dephosphorylation. However, only 2 out of >20 NF200-specific MAs recognize the modified polypeptide form. Of >60 other MAs that recognize NF200, but also cross-react with NF150, <5% recognize these polypeptides in dephosphorylated form. Two of 4 NF150-specific MAs react with NF150 after dephosphorylation. MAs that do not recognize NF200 or NF150 after phosphatase treatment are mostly (>95%) directed against the portions of these polypeptides that are released from the NF 'backbone' by limited chymotryptic proteolysis.

We conclude that phosphorylation significantly affects immunogenicity of NF200 and NF150, possibly by determining the conformation of these polypeptides in NFs.

308.12 Ca^{++}CALMODULIN DEPENDENT PHOSPHORYLATION IN IMMATURE RAT CEREBRAL CORTEX UNDERGOING HYPOXIA-ISCHEMIA. C.L. Powell*, A.M. Morin and C.G. Wasterlain*. Neurology Research Lab., Vet. Admin. Med. Ctr., Sepulveda, CA 91343.

Some of the biological effects of Ca^{++} in brain may be mediated by Ca^{++} calmodulin (CaCM) dependent protein phosphorylation (DPP). Because of CACM's role in neurotransmission and because hypoxia-ischemia (HI) is a common precursor of neuronal injury and seizures in immature brain, we sought to study the effects of HI on CaCM DPP of the 50, 58, and 60 K subunits of CaCM kinase II in the newborn rat brain. Six day old rats (n=10) underwent left carotid ligation under light anesthesia. Following a 3 hour rest period they were exposed to 2.5 hours of 8% O_2 and 92% N_2. Thereafter the pups were sacrificed at varying intervals: t=0 hours (n=3), t=2.5 hours (n=3), t=24 hours (n=2), and t=48 hours (n=2). Non-ligated normoxic rats (n=9) at 6d, 7d, and 8d each and ligated normoxic rats (n=3) served as controls. The left and right cortices were dissected, homogenized, and centrifuged for isolation of synaptosomal fractions. The phosphorylation assay included 0.6mM Ca^{++}, calmodulin, 10.0mM Mg^{++}, 0.5mM EGTA and [γ-^{32}P] ATP as phosphate donor. Proteins were subjected to SDS-polyacrylamide gel electrophoresis. Gels were then exposed to x-ray film for autoradiographic localization of phosphoprotein bands. The 50, 58, and 60 K bands showed no change in CaCM stimulation from control at t=0 but inhibition greater on the ligated side at t=2.5 hours. Ligated animals dying during the hypoxia also had partial inhibition with CaCM on these bands. By t=24 hours, there was partial stimulation by CaCM of the 3 bands, greater on the non-ligated side. At t=48 hours, recovery was complete for the 50 K band but partial for the 58-60 K doublet with less stimulation by CaCM on the ligated side. Ligated normoxic animals showed no relative change in CaCM DPP. These preliminary results suggest that the 50, 58, and 60 K subunits of the CaCM kinase II complex may be markers of ischemic cell damage in the newborn rat brain. Unlike the classical markers (e.g., ATP) which quickly recover once O_2 is restored, longer inhibition of this enzyme complex was observed. Since CaCM DPP may affect such diverse processes as the polymerization of tubulin and may modulate neurotransmitter release from synaptic vesicles, the inhibition observed in the HI immature brain may suggest a mechanism for abnormal neurotransmission and seizures in the high-risk neonate. (This work was supported in part by DHHS funds under federal contract #NO1-NS-0-2332 and by the Epilepsy Foundation of America Grant #P821228.)

308.13 PROTEIN CARBOXYL METHYLTRANSFERASE PREFERENTIALLY MODIFIES ISOASPARTYL RESIDUES IN DEAMIDATED BRAIN PROTEINS. B.A.Johnson*, N.E.Freitag* and D.W.Aswad. Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

Protein carboxyl methyltransferase (PCM) has been assumed to play a role in cellular stimulus-response coupling. However, even its best protein substrates appear to accept substoichiometric numbers of methyl groups. The substrate porcine adrenocorticotropin (ACTH-Sigma) accepted only 0.03 mol CH_3/mol peptide. The low methyl incorporation reflected a subpopulation of $_{25}$ACTH molecules. ACTH is known to become deamidated at Asn^{25}. Its lability is derived from the presence of an Asn-Gly sequence. Asn-Gly sequences facilitate the deamidation of the Asn because of their tendency to form a cyclic imide intermediate. Ring-opening results in proteins with either α- or β-carboxyl linked Asp, with the latter, isoaspartate, form predominating (Bornstein,P. and Balian,G., Meth. Enzymol., 47, 132-145, 1977).

ACTH was deamidated by alkaline treatment, and the deamidated species was isolated. Deamidated ACTH accepted 0.78 mol CH_3/mol peptide from PCM, which is consistent with the stoichiometric methylation of the α-carboxyl group of isoaspartate. This is the highest stoichiometry of carboxyl methylation yet reported. Increases in the stoichiometry of methylation following alkaline treatment were also seen for calmodulin (0.02→0.39 mol %), prolactin (0.08→0.24 mol %), and synapsin I (0.04→0.68 mol %), but not for lutropin. Calmodulin and prolactin contain Asn-Gly sequences; lutropin does not. At pH 7.4, 37°C, methylated ACTH became rapidly demethylated to a form not capable of accepting methyl groups from PCM. Demethylation resulted in the regeneration of the cyclic imide. Hydrolysis of the isolated imide resulted in the recovery of substrate which can be methylated to the same stoichiometry as deamidated ACTH.

These results may explain two well-known paradoxes of mammalian protein carboxyl methylation. Substoichiometric methylation of proteins may reflect the methylation of a subpopulation containing isoaspartate. The extreme instability of the protein methyl esters may be caused by the rapid formation of a cyclic imide following esterification of isoaspartate in an Asp-Gly sequence. The results suggest that carboxyl methylation may have a role either in the repair of age-deamidated proteins or in the activation of isoaspartyl-like α-carboxyl groups for nucleophilic attack by some other molecule.

308.14 CALMODULIN-DEPENDENT MYOSIN LIGHT CHAIN KINASE FROM BOVINE BRAIN. D.C. Bartelt*, S. Moroney* and D.J. Wolff*. (Spon. H.M. Geller). Dept. of Pharmacology, UMDNJ-Rutgers Medical School, Piscataway, N.J. 08854

In the course of our characterization of calmodulin-binding proteins in bovine brain, we have detected the presence of a calmodulin-dependent protein kinase capable of phosphorylating the M_r 20,000 myosin light chain isolated from chicken gizzard. The kinase is prepared from a post-microsomal supernatant fraction by chromatography on phosphocellulose. Greater than 90 percent of the calmodulin-dependent myosin light chain kinase (CaM-MLCK) is bound by the resin in 30 mM Piperazine-N,N'-bis[2-ethanesulfonic acid] (PIPES) pH 6.9, and 65 percent is recovered by elution with 0.15 M NaCl. The enzyme is free of cAMP-dependent protein kinase and calcineurin, the calmodulin-dependent protein phosphatase. CaM-MLCK is further purified by affinity chromatography on calmodulin-Affi-Gel. At this stage of purification, phosphorylation of myosin light chain is stimulated 350 fold by the presence of calcium/calmodulin. CaM-MLCK exhibits K_m values of 4.8 µM for myosin light chains and 16 µM for ATP. In addition to smooth muscle myosin light chain, CaM-MLCK phosphorylates rabbit liver glycogen synthase but exhibits less than one-tenth the activity towards this substrate as compared with myosin light chain. CaM-MLCK does not phosphorylate skeletal muscle myosin light chain, myelin basic protein, histones 1 and 2b, casein, phosphorylase or microtubule-associated protein 2 to any appreciable extent when measured at a final substrate concentration of 300 µg/ml.

CaM-MLCK differs in substrate specificity from Ca^{2+}/calmodulin kinase II isolated from rat brain by several laboratories. CaM-MLCK is similar in substrate specificity to bovine brain Ca^{2+}/calmodulin kinase I (Nairn and Greengard, Abstr. Soc. for Neurosci. 9, 1029, 1983); however the molecular weight of native Ca^{2+}/calmodulin kinase I is reported to be 46,000. The molecular weight of CaM-MLCK is estimated at 270,000 by gel filtration on Sepharose 4B. CaM-MLCK appears to be distinct from any protein kinase previously reported. (Supported by N.I.H. grant NS 11252 and UMDNJ-Rutgers Medical School General Research Support Fund.)

308.15 A NEURON SPECIFIC MONOCLONAL ANTIBODY RECOGNIZING A SMALL NUCLEAR PROTEIN IN RAT BRAIN. S.J. Hapner and C.M. Paden. Dep't. of Biology, Montana State University, Bozeman, Mt.59717

During the course of experiments designed to produce monoclonal antibodies (MABs) to membrane antigens of neurosecretory cells, we have generated a MAB (designated F5-26) which labels cell nuclei of neurons throughout the CNS. F5-26, an IgG, was generated against a crude membrane fraction from the paraventricular nucleus of the hypothalamus using a combination of a priming immunization and a secondary in vitro activation containing 80µg of protein from a β-octyl glucoside extract of the original membrane preparation. The antibody secreting hybridoma was cloned twice by limiting dilution, and passed through an ascites tumor to produce large quantities of antibody. Immunocytochemical characterization of F-5 26 was performed at a 1:500 dilution on 50µVibratome sections following perfusion of adult rats with a modified Zamboni's fixative.

Immunoperoxidase labelling of rat brain (PAP method) revealed that F5-26 stained the nuclei of virtually all neuronal types in the CNS. The nucleolus was unstained, as were glial nuclei. Although the precise localization of the nuclear antigen has not been determined, the staining pattern indicated that it might be located on the nuclear membrane. In addition to this nuclear labelling, a generally fainter and more variable staining of neuronal cytoplasm was apparent. Preliminary evidence suggests that F5-26 labeling is nervous system specific. Anterior pituitary, liver, cardiac and skel etal muscle showed no immunoreactivity in either cell nuclei or cytoplasm. The peripheral nervous system has not yet been examined.

Specificity of F5-26 was further characterized by SDS polyacrylamide slab gel electrophoresis and immunoblotting. Analysis of whole brain homogenate revealed the presence of 3 bands of approximate molecular weights 41,700, 26,700, and 12,500. When a nuclear fraction from whole brain homogenate was isolated by differential centrifugation only the 12,500 dalton band was detected. These results suggest that F5-26 recognizes a single nuclear protein of small size which appears to be restricted to neurons. Such a protein could play a role in regulation of neuronal gene expression. Further characterization of this MAB will be useful in determining if it is indeed CNS specific and in exploring the relationships between the nuclear and cytoplasmic proteins which it recognizes.

This work was supported by NIH NS17974 and a Grant-in-Aid from the Montana Heart Association.

308.16 CHARACTERIZATION OF FETAL CALF SERUM CHOLINESTERASE. Paul M. Salvaterra and Margaret G. Filbert*. (SPON: P. Lynn Donaldson) Div of Neurosci., City of Hope Rsch Inst, Duarte, CA 91010 and US Army Med Rsch Inst of Cml Def, Aberdeen Proving Ground, MD 21010

Fetal calf serum (FCS) is used widely to supplement tissue culture media for neurogenic and myogenic cell lines as well as for primary cultures. Since many studies require the determination of acetylcholinesterase (AChE) activities in cultured cells, the hydrolysis rates of acetyl- -methylcholine (MeCh) and butyrylcholine(BuCh),specific substrates for AChE and BuChE (pseudocholinesterase), were measured in FSC with the radiometric assay of Siakotis et al. (Biochem Med 3:1, 1969). The rate of ACh hydrolysis by the FCS enzyme was used as a control. These were compared with hydrolysis rates by horse serum, another widely used media supplement that contains primarily BuChE.

SUBSTRATE	Hydrolysis Rate DPM/min/mg protein	% Compared to ACh Hydrolysis
ACh		
Horse serum	313.5	100
FCS	284	100
MeCh		
Horse serum	9.6	3
FCS	83.4	30
BuCh		
Horse serum	799.6	255
FCS	44.5	16

To characterize the esterases further, the ability of specific inhibitors to block the hydrolysis of ACh by FCS was examined. At 10^{-7}M, 1,5-bis(4-allyldimethylammonium-phenyl)pentane-3-one (BW284C51), a selective inhibitor of AChE, (Austin & Berry, Biochem J. 54: 695, 1953) inhibited the FCS enzyme 96%. Tetraisopropyl pyrophosphoramide (iso-OMPA), an irreversible inhibitor of BuChE, (Bayless & Todrick, Biochem J. 62:67, 1956) had no effect on the FCS enzyme until the concentration was increased to 4×10^{-5}M. This concentration of iso-OMPA inhibited FCS esterase activity by only 20%. These results indicate that FCS contains predominately AChE. Therefore, care should be taken to remove FCS by thorough washing if an accurate measurement of AChE activity in cultured cells is desired.

309.1 EFFECTS OF D(+)-AMPHETAMINE ON BLOWFLY FEEDING BEHAVIOR. R.S. Edgecomb*, T.F. Long*, L.L. Murdock. Dept. of Entomology, Purdue University, West Lafayette, IN 47907.

Recent studies in our laboratory into the biochemical and neural mechanisms of adult black blowfly (Phormia regina Meigen) feeding behavior have indicated that aminergic neurotransmitter systems (octopamine, dopamine, and serotonin) participate in the control of feeding (Long, T.F. and Murdock, L.L., PNAS, 80:4159, 1983). We have observed that D(+)-amphetamine, a well-studied anorectic in mammals, affects blowfly feeding behavior in at least two ways. In starved flies, injections of D(+)-amphetamine (10 µg) caused a marked decrease in the proboscis extension response (PER) to tarsal stimulation with aqueous sucrose solutions while general behavior appeared unchanged. Typically, mean acceptance thresholds rose from 10 mM sucrose to over 500 mM. In contrast to the effect on PER, D(+)-amphetamine (10 µg) caused a slight but significant increase in the amount of 1 M sucrose consumed by hungry flies. Higher doses caused greater increases in meal size. Electrophysiological studies determined that injected D(+)-amphetamine (10 µg) and related compounds had no significant effect on tarsal sugar receptor activity. In view of the well-established effects of D(+)-amphetamine on aromatic biogenic amine systems in mammals, the present observations provide additional support for an involvement of such amines in the central control of adult blowfly feeding behavior.

309.2 THYROIDECTOMY AND IMIPRAMINE TREATMENT IN THE RAT: A 2-DEOXYGLUCOSE STUDY. E.H. Elowitz*, L.A. Freed*, J.W. Melisi*, and D.L. Dow-Edwards. Lab. of Cerebral Metabolism, Dept. of Neurosurgery, SUNY, Downstate Medical Center, Brooklyn, NY 11203.

Both altered thyroid hormone levels and the tricyclic antidepressant drug, imipramine, have been shown to affect central neural function. Although various studies have examined the effects of hypothyroidism and imipramine on neurochemistry, little is known of the metabolic response of specific brain structures. In order to elucidate the effect of altered thyroid status and imipramine therapy on brain function in general, and monoaminergic systems in particular, we chose the quantitative autoradiographic 2-deoxyglucose (2DG) method which measures glucose utilization in brain (Sokoloff, L. et al., J. Neurochem., 28:897-916, 1977).

Four groups of animals were designated: (1) sham-operated + saline inject; (2) thyroidectomy (TX) + saline inject; (3) sham-operated + imipramine inject; (4) TX + imipramine inject. Male Sprague-Dawley rats (300-400 gms) were subjected to halothane-N_2O anesthesia and either thyro-parathyroidectomy or sham operation. Fourteen days after surgery, daily IP injections of either 10 mg/kg imipramine in saline or 0.5 ml/kg saline were begun. Following two weeks of injections, the 2DG method was performed. Catheters were inserted into the femoral artery and vein of each rat under halothane-N_2O anesthesia. ^{14}C-labeled 2DG was injected into the venous catheter. Timed arterial blood samples were collected. At 45 minutes, the animals were killed with pentobarbital. Local cerebral glucose utilization was then determined from autoradiographic analysis and the integrated arterial curve for 2DG and glucose.

Data were analyzed using ANOVA techniques. Significant alterations in several regions occurred. An interaction between TX and imipramine was found in the central amygdaloid nucleus, the lateral habenula and the supraoptic nucleus of the hypothalamus. TX alone reduced glucose utilization in the motor and auditory systems, the hippocampus, the parietal cortex and the pontine reticular formation. Imipramine alone reduced glucose utilization in the median eminence.

309.3 THE SPONTANEOUS SEIZURE GENOTYPE SZ IN HAMSTERS AS A DISEASE MODEL OF BENZODIAZEPINE RECEPTOR DEFICITS. J.E. Fisher, Jr.* and W. B. Iturrian. Pharmacology Dept., University of Georgia, Athens, GA 30602.

We propose the spontaneous seizures (sz) which occur in BIO 86.93 mutant hamsters (J. Heredity 67:115,1976) as a disease model produced by alterations in endogenous benzodiazepine (BDZ) ligands and/or their receptors. These genetically determined compulsive sets of reproducible abnormal motor activities with distinctive stereotyped behavior and myoclonic seizures appear during mild anxiety provoking situations (eg. weaning). The trait is an autosomal simple recessive genetic mutation that produces highly predictable incidence of spontaneous seizures in offspring from classical genetic breeding studies. The hybrids afford large litters and excellent reproduction potential (Ga. J. Sci. 42:45,1984). A seizure score severity was developed as the phenotypically distinguished seizure occurs in definable stages of: hyperkinetic-ataxic gait, straub-tail, falling, behavioral "absence", tonic hindlimb spasms, wading pool crawl, steriotypic head and psychomotor movements, followed by a paralytic state of extended rigid hindlimbs with myoclonus of forelimbs and jaw. This distinctive sz seizure can be elicited with an anxiogenic dose of pentylenetetrazol (PTZ) or picrotoxin which are never convulsive. Phenytoin also exacerbates sz, but even convulsant doses of PTZ does not produce sz type of seizure unless the animals are homozygous for the mutation.

We suggest deficits in endogenous inhibitory systems in the sz hamster as demonstrated by lack of postictal depression in the paired maximal electroshocks method of Freston and Esplin (Exp. Neurol. 4:221, 1961). Maximal electroshock is reported (Science 202:892,1978) to increase the number of available BDZ binding sites and we find it prevents sz seizure with a temporal course resembling the receptor effect.

CGS 8216 and βCCE are antagonists with high BDZ binding but devoid of overt activity in normal animals. These compounds initiate sz in homozygotic hamsters suggesting an involvement of the high affinity BDZ ligand-receptor system in the sz seizure. Both the spontaneous and drug induced sz seizure are blocked by the BDZ agonists nordiazepam or CGS 9896. Since this mutation has relevance for both anxiety and seizures it offers an exciting new model for studying the behavioral significance of deficits in the endogenous benzodiazepine ligands and/or their receptors.

309.4 COPING AND SEIZURE SUSCEPTIBILITY: CONTROL OVER STRESS PROTECTS AGAINST SEIZURES. R.C. Drugan, T.D. McIntyre, H.P. Alpern, and S.F. Maier. Department of Psychology, Box 345, University of Colorado, Boulder, CO. 80309

Control versus lack of control over stress has been demonstrated to differentially affect behavior, physiology, and stress symptoms elicited by the same stressor. Research has been conducted to ascertain the endogenous changes responsible for the deficits following inescapable shock and various neurotransmitter systems have been implicated (NE, 5-HT, ACh, GABA). However, little research has been done concerning the endogenous mechanism(s) responsible for the protective effects of stress control. Petty and Sherman have shown that inescapable shock produces a decrease in calcium-induced release of GABA, while an injection of GABA prevents some of the effects of inescapable shock. Indirect evidence with benzodiazepines and β-carbolines also suggest that GABA is important. Librium given before inescapable shock prevents the analgesia and escape deficit, while an anxiogenic β-carboline (FG-7142) given in lieu of inescapable shock produces a comparable escape deficit (Drugan et al. 1983; Drugan et al. 1984).

In sum, both GABA and benzodiazepines which are known to facilitate GABAergic transmission protect against inescapable shock-induced deficits, while both GABA antagonists (bicuculline) and benzodiazepine inverse agonists (FG-7142) known to attenuate GABAergic transmission induce a behavioral syndrome comparable to that produced by inescapable shock. These data suggest that GABA plays a critical role in modulating the differential impact of escapable and inescapable stress.

To test our hypothesis that control over or coping with a stressor facilitates GABAergic transmission, we employed a behavioral index of GABA levels in the CNS. We report that the latency to bicuculline-induced clonic seizures is differentially affected by control/lack of control over shock. Rats given 80 escapable shocks showed increased latency to seizure 2 hours later, while inescapably shocked rats demonstrated a marked decrease in seizure latency. In addition, we confirmed the previous finding that acute inescapable shock (20 shocks) protects against seizures both immediately and 2 hours later, while chronic (80) inescapable shock protects against seizures if tested immediately post-stress. We suggest that coping with stress facilitates GABAergic transmission which has a prophylactic effect on many stress indices.

309.5 GABA/BENZODIAZEPINE MODULATION OF THE PROCONVULSIVE EFFECT OF UNCONTROLLABLE STRESS. T.D. McIntyre, R.C. Drugan, S.F. Maier, and H.P. Alpern. Behavioral Neuroscience Program and Department of Psychology, University of Colorado, Boulder, Colorado, 80309.

We have reported (this volume) that the control an animal exerts over stressful situations critically influences seizure latencies. Specifically, rats given 80 escapable shocks showed increased latency to bicuculline-induced seizures, while inescapably shocked animals exhibited a significant decrease in latency to clonic seizures. Sherman and Petty (1981) have shown that inescapable shock elicits a decrease in Ca^{++}-evoked GABA release from the hippocampus. Further, indirect evidence indicates that the benzodiazepine (BZ) receptor may also influence the outcome of inescapable stress. Chlordiazepoxide prevents the subsequent analgesia and escape deficits produced by inescapable stress, while the β-carboline FG-7142 produces deficits comparable to inescapable stress. In summary, our results, as well as those of others, implicate the GABA-BZ complex in modulation of the effects of escapable and inescapable stress.

Much evidence suggests that GABA is the prime regulator of epileptic activity. Thus, agents interfering with GABA transmission directly, such as bicuculline, or indirectly, such as the BZ antagonist β-CCM, induce seizure activity. Conversely, agents which elevate GABA levels, such as AOAA, have an anticonvulsant effect (Greer and Alpern, 1978). The imidazodiazepine RO 15-1788 was originally thought to be a specific BZ antagonist without any intrinsic activity (Mohler et al, 1981), but has since been shown to have partial agonist activity, i.e., it blocks pentylenetetrazol- and bicuculline-induced seizures (Nutt, 1983).

If our hypothesis concerning the GABA-BZ mediation of the effects of escapable and inescapable stress is valid, then it would be of interest to ascertain the effects of AOAA and RO 15-1788 on the proconvulsive effects of inescapable shock. We report that both AOAA and RO 15-1788 antagonize the proconvulsive effects of inescapable shock. Further work delineating these mechanisms is currently underway.

We would like to thank Hoffman-La Roche for their generous gift of RO 15-1788.

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309.6 EFFECTS OF PARA-CHLOROPHENYLALANINE (PCPA) ON PAIN THRESHOLDS AND INDOLE LEVELS IN RAPHE NUCLEI AND SPINAL CORD. J.L. Steinman, S.M. Carlton, B. Haber and W.D. Willis. Marine Biomed. Inst. and Depts. of Anatomy and of Physiol. & Biophys., Galveston, TX 77550-2772.

The involvement of endogenous serotonergic pathways in the mediation of antinociception has been indicated by electrophysiological, pharmacological and behavioral experiments. However, manipulation of the indole pathway, either by lesioning of raphe nuclei or drug intervention, often produces disparate results. In particular, PCPA, administered to inhibit 5HT synthesis by inactivating the rate limiting enzyme tryptophan hydroxylase, has been reported to produce either hyperalgesia or analgesia, depending upon the type of pain measurement examined. In the present study, we sought to correlate the effects of PCPA on 1) behavioral responses to noxious stimulation and 2) direct measurements of 5HT, tryptophan and 5HIAA in raphe nuclei (pallidus, obscurus, magnus and dorsalis) and spinal cord by HPLC-EC detection.

Before drug treatment, baseline tail flick latencies (TFL) to radiant heat and vocalization thresholds (VT) to electric shock of the tail were measured. For 3 consecutive days, rats were injected IP with 200, 400 or 600 mg/kg PCPA. On day 4, TFL and VT were determined and rats sacrificed. Brains and lumbar spinal cord were immediately removed, frozen in liquid N_2 and stored at -70°C. Serial coronal brain slices of 300 μm were cut and raphe nuclei punched out with stainless steel needles (15-23 ga). Samples were homogenized in 0.05M perchloric acid and centrifuged on the day of HPLC analysis.

PCPA elevated both TFL and VT in a dose-dependent manner. In the 600 mg/kg group, none of the subjects vocalized at any current intensity (to 4mA), although vocalization could be elicited by innocuous stimulation (rapid lifting out of cage). The 5HT levels did not show an unequivocal dose-dependent reduction.

The data indicate that even at the highest dose of PCPA, differential amounts of 5HT remain that could be released upon activation of serotonergic structures by particular nociceptive stimuli. Preliminary measurements of tryptophan and 5HIAA suggest that PCPA has differential effects on synthesis in different regions. (Supported by NS11255, NS09743, NS17696 and NS07022.)

309.7 **MYOCLONIC JUMPING BEHAVIOR: A POTENTIAL ANIMAL MODEL FOR SCREENING HALLUCINOGENIC AGENTS** P.A. Nausieda, R. Weerts*, P. Carvey*, Neurology Research Dept. V. A. Medical Center Wood, WI 53193

Myoclonic Jumping Behavior (MJB), a clonic retroflexion of the forelimb and hindlimb musculature, is a behavioral response first observed following the administration of 5-hydroxytryptophan in guinea pigs. In an effort to determine whether or not d-lysergic acid diethyamide (LSD) was capable of potentiating myclonic responsiveness it was observed that this agent alone induced the behavior. This finding suggested that if other hallucinogenic agents induced MJB, this behavior could serve as an animal model for screening hallucinogenic agents.

There are four characteristics of the human hallucinogenic experience which should act as criteria for this animal model: (1) that the behavioral index used to score the activity of the hallucinogenic agent should vary in a dose dependent fashion, (2) that repeated administration of the agent should induce rapid tachyphylaxis, (3) that a cross-tolerance should exist between known hallucinogenic agents, and (4) that the behavioral response to a series of hallucinogenic agents of varying potency should be consistent with that found in humans.

Studies with d-lysergic acid diethylamide (LSD) have yielded positive results with respect to the first three criteria. Twelve groups of guinea pigs were administered doses of LSD at 2.5, 5, 10, 25, 50, 100, 250, 350, 500, 1000, 1500, and 2000 mcg/kg. Statistical analysis indicates a near linear relationship between the frequency of MJB and the dose level of LSD ($p<0.001$). Five groups of animals administered repeated doses of LSD at 50, 150, 250, 350, and 500 mcg/kg on three consecutive days demonstrated a significant decrease in MJB for each dose level on each day ($p<0.001$). Cross-tolerance of LSD to mescaline (MES) was demonstrated using two groups of six guinea pigs. One group was administered a dose of 50 mg/kg MES; the other an equal dose/kg of normal saline per day for five consecutive days. Challenged on the sixth day with LSD (500 mcg/kg) the MES treated group exhibited a significantly lower response rate than the saline controls ($p<0.001$).

Preliminary work with four other agents known to induce hallucinosis in humans; (dl)-2,5-dimethoxy-4-methylamphetamine (DOM), (dl)-2,5-dimethoxyamphetamine (DMA), (dl)-3,4,5-trimethoxyamphetamine (TMA), and mescaline, suggests that they also meet the first three criteria and taken together with LSD exhibit a variation in potency which is consistent with that found in humans. Further investigations are presently being carried out which would extend the model elaborated for LSD to the these other hallucinogins. If, as our preliminary data suggests, these agents fulfill the necessary criteria MJB, could serve as a reliable, inexpensive method for assessing the hallucinogenic potential of pharmacologic agents.

309.8 SUBSTANCE P AND SUBSTANCE K, COTRANSMITTERS DERIVED FROM A COMMON PRECURSOR, HAVE SIMILAR POSTSYNAPTIC ACTIONS MEDIATED BY SEPARATE RECEPTORS. J.F. Bishop, C.W. Shults and T.L. O'Donohue. Experimental Therapeutics Branch, NINCDS, Bethesda, MD 20205

Two genes encoding substance P (SP) precursors have recently been identified from bovine striatum (Nawa et al. Nature, 306:32, 1983). One precursor contains only SP while the other contains SP and substance K (SK), a peptide named for its structural similarity to kassinin. This SP/SK precursor appears to be processed to SP and SK as both peptides can be measured in rat brain and have a parallel regional distribution (Shults et al., Soc. Neurosci., 1984). Furthermore, separate binding sites for 125-I-SP and 125-I-SK, labeled by the Bolton-Hunter method, are found in the brain (Shults et al., Soc. Neurosci., 1984) and in the periphery (Burcher et al., Soc. Neurosci., 1984). The purpose of the present study was to determine if separate receptors for SP and SK exist in the spinal cord and to compare the behavioral actions of intraspinal injections of SP, SK and kassinin. In the rat spinal cord, SP receptors have been identified in the dorsal horn using autoradiography with Bolton-Hunter labeled 125-I-SP (Quirion et al., Nature, 303:714, 1983; Shults et al., Peptides, 3:1073, 1982). In the present study, SK binding sites were also found in the spinal cord using Bolton-Hunter 125-I-SK. SK binding sites were primarily localized in the superficial laminae of the dorsal horn as were SP receptors. In the second part of the present study, the postsynaptic actions of SP, SK, and kassinin were studied using a modification of a behavioral testing procedure (Piercy et al., Brain Res. 210:407, 1981). In this procedure, intraspinal injections of SP are thought to mimic the effects of cutaneous irritation by stimulating target cells of primary sensory afferents, producing bite/scratch behavior. Briefly, male mice received intraspinal injections of either SP, SK, kassinin, or vehicle (0.01 M HAc), and bite/scratch behavior was assessed. Bite/scratch behavior was judged to be either present or absent after doses of peptide ranging from 10 to 1000 picomoles, and the results were expressed as the fractional number of mice responding. SP, SK and kassinin all produced bite/scratch behavior characterized by brief episodes of biting, licking, or scratching primarily directed at the hindquarters. The three peptides were approximately equipotent in eliciting this behavior, with ED50s ranging from 10 to 30 picomoles. Injections of vehicle had no effect on bite/scratch behavior. The results of the two parts of this study suggest that SP and SK, two peptides derived from a common precursor, have similar postsynaptic actions which may be mediated by separate receptors. The results of this study are also consistent with recent findings showing similar electrophysiological actions of SP and SK in the midbrain (Lanthorn et al., Soc. Neurosci., 1984).

309.9 SENSITIZATION, FEAR CONDITIONING, AND PHARMACOLOGICAL MODULATION OF ACOUSTIC STARTLE FOLLOWING LESIONS OF THE DORSAL PERIAQUEDUCTAL GRAY. J.V. Cassella and M. Davis. Dept. of Psychiatry, Yale Univ. Sch. of Med. New Haven, CT 06508.

Lesions of the dorsal periaqueductal gray (DPAG) have previously been found to produce both a generalized hyperresponsiveness to sensory stimulation and transient explosive motor behavior in the rat. The acoustic startle response is useful in measuring sensory-motor reactivity since it is a short-latency reflex mediated by a relatively simple neural pathway. Recent anatomical evidence has revealed that the DPAG projects to the primary startle pathway. Thus, it is possible that the DPAG modulates the acoustic startle response. The present series of experiments examined the effects of electrolytic lesions of the DPAG on acoustic startle and also explored the effects of these lesions on the action of drugs previously reported to modify the amplitude of the startle response.

Rats were lesioned bilaterally with two A-P placements in the DPAG (.1 mA DC anodal current for 60 sec; coordinates of Paxinos and Watson, 1982 : A-P = -5.8 and -6.8 relative to Bregma; M-L = ± 0.5 ; D-V = -5.5) or were sham operated. Testing was conducted when the explosive motor behavior had disappeared.

Lesioning the DPAG dramatically increased startle amplitude. Despite their very high startle levels, lesioned animals still showed potentiated startle when the startle-eliciting stimulus was presented in the presence of a light that had previously been paired with a shock, and displayed normal pre-pulse inhibition. These animals habituated early in the test session but showed exaggerated sensitization later in the session. The typical excitatory actions of the DA agonist, apomorphine, and the glycine antagonist, strychnine, were observed in lesioned animals. The alpha-2 adrenergic agonist, clonidine, still decreased startle amplitude in these rats. The excitatory and inhibitory effects of these drugs in lesioned animals paralleled those of sham animals but were superimposed on a markedly higher startle baseline.

In summary, the DPAG exerts a strong functionally-inhibitory effect on processes involved in sensitization of the acoustic startle reflex but does not mediate alterations in startle produced by several behavioral and pharmacological manipulations. Further studies will determine the exact nature of this potent modulatory action of the DPAG on the acoustic startle response.

309.10 EFFECTS OF AGONISTS AND ANTAGONISTS OF D1 AND D2 DOPAMINE RECEPTORS ON DYSKINETIC MOVEMENTS ASSOCIATED WITH IDPN INTOXICATION. A.Y. Oda* and J.S. Schneider (SPON: F.J. Denaro). Mental Retardation Research Ctr., UCLA, Los Angeles CA 90024.

IDPN (3'3'-iminodiproprionitrile) intoxication has been shown to produce in rats a complex motor disorder consisting of locomotor hyperactivity, circling, and choreo-athetoid head movements. Components of this syndrome, particularly the abnormal head movements, have been shown to be related to basal ganglia functions (Schneider, Exptl. Neurol., in press) and to respond to manipulations of the dopaminergic system. Presently, we have examined the possible participation of D1 versus D2 dopamine (DA) receptors mediating abnormal involuntary head movements associated with the IDPN-induced syndrome.

After a baseline frequency of abnormal head movements was determined, rats were randomly assigned to various treatment groups. Chlorpromazine (0.1 mg/kg) and cis-flupenthixol (5, 10 mg/kg) were used as D1-D2 receptor antagonists, sulpiride (1,10,20 mg/kg) and haloperidol (.1,12 mg/kg) as D2 receptor antagonists and SCH 23-390 (.1,2.5,10 mg/kg) as a specific D1 antagonist. Apomorphine was used as a D1-D2 agonist (2.5, 5 mg/kg), and lisuride (.05,.1,.2 mg/kg) and bromocryptine (1,2,8 mg/kg) as predominantly D2 agonists. All compounds caused some reduction in the frequency of abnormal head movements. However, D1 and D1-D2 antagonists were more effective than D2 antagonists alone. Some of these effects were dose-dependent. While D1 or D2 receptors alone do not appear to be exclusively involved in the observed movement disorder, there may be some preferential involvement of D1 receptors. This complex motor disorder, whose pathophysiology is unknown, may to some extent involve an alteration of mutual equilibrium of D1 and D2 receptors. Reduction in frequency of head movement observed with DA agonists could be related to activation of presynaptic DA receptors in the substantia nigra and striatum (thus inhibiting DA release) or of postsynaptic DA receptors. The effect could also in part be related to postsynaptic blockade of DA receptors by partial agonistic effects of the compounds used. The inhibitory effect on movement seen with lisuride may additionally be due in part to its high affinity for serotonin as well as for DA postsynaptic receptors. Lisuride also had a more potent inhibitory effect on movement than did bromocriptine, although both are ergot D2 agonists. These results indicate the pharmacological complexity of this syndrome, which is in part demonstrated by both agonists and antagonists of DA receptors producing similar effects on the motor disorder. (Supported by NIH Grant RR05756)

309.11 REDUCTION OF HEAD SHAKE BEHAVIOR AND $5HT_2$ RECEPTORS IN RATS TREATED REPEATEDLY WITH ANTIDEPRESSANT DRUGS. I. Lucki, H.R. Ward*, L.S.Y. Tyau* and A. Frazer. Departments of Psychiatry and Pharmacology, University of Pennsylvania, VA Medical Center, Philadelphia, PA 19104.

The repeated administration of various types of antidepressant drugs to rats result in a reduction in the number of $5HT_2$ receptors in rat frontal cortex (Science, 210:88, 1980). This study examined whether head shake behavior in rats produced by quipazine, a behavioral response associated with $5HT_2$ receptors (J. Pharmacol. Exp. Therap. 228: 133, 1984), is altered by antidepressant treatments that produce reductions in the number of $5HT_2$ receptors.

The head shake response is defined as a rapid lateral twitch of the head. Quipazine (0.5-8mg/kg) was injected i.p. to induce head shake behavior and the number of head shakes were counted for the next 30 minutes. Separate groups of rats were treated with the monoamine oxidase inhibitors (MAOIs) nialamide (40mg/kg/day) or phenelzine (10 mg/kg/day), the tricyclic antidepressants desipramine (10 mg/kg, bid) or amitriptyline (10mg/kg, bid), or the atypical antidepressant drug iprindole (10mg/kg, bid) for seven days. Rats were examined for head shake behavior 24 hours after the final injection. Separate groups of rats were treated similarly with the antidepressant drugs for seven days and sacrificed 24 hours after the final injection in order to examine the number of $5HT_2$ receptors in rat frontal cortex, using ^{3}H-spiroperidol as a ligand.

Treatment with antidepressant drugs for seven days resulted in a significant attenuation of the head shake response to quipazine and a reduction in $5HT_2$ receptors in rat frontal cortex. Nialamide and phenelzine were the most effective drugs at reducing head shake behavior, producing about an 85-95% blockade of quipazine's ability to elicit the behavior. The tricyclic antidepressant desipramine and amitriptyline were less effective at reducing head shake behavior than the MAOIs, and iprindole's effect was intermediate between the two classes of drugs. All of the antidepressant drugs produced a significant reduction in ^{3}H-spiperone binding.

The chronic administration of antidepressant drugs, which cause a reduction in the number of $5HT_2$ receptors in rats frontal cortex, also produce a reduction in a behavioral response, head shake behavior, that is associated with $5HT_2$ receptors. (Supported by NIHM grant MH 36262 and funds from the Veterans Administration.)

309.12 THE CONCURRENT ADMINISTRATION OF ANTIMUSCARINIC AGENTS REDUCES THE DEGREE OF BEHAVIORAL HYPERSENSITIVITY INDUCED BY HALOPERIDOL. Paul M. Carvey*, L.C. Kao*, C. Goetz*, C. Tanner* and H.L. Klawans*(SPON: Ana Hitri). Depts. of Neurological Sciences and Pharmacology, Rush-Presbyterian-St. Luke's Med. Center, Chicago, IL 60612

Animals treated with neuroleptic agents exhibit an increased behavioral response to DA agonists upon their withdrawal from chronic treatment. This behavioral hypersensitivity (BH) is correlated with and is thought to result from the proliferation of DA receptors within the striatum. At these meetings two years ago, we reported that the degree of BH associated with the chronic treatment of equivalent doses of various neuroleptic agents was inversely proportional to their antimuscarinic potency. We have recently completed a series of experiments which investigated this relationship by coadministering three different antimuscarinic agents with haloperidol (HAL), a neuroleptic agent considered devoid of antimuscarinic activity.

Guinea pigs and rats were treated with HAL along with various doses of trihexyphenidyl (TRI), scopolamine or benztropine for 24 consecutive days. They were then withdrawn from treatment and challenged with apomorphine (APO) 96 hours later. HAL significantly increased stereotypic responsiveness. The coadministration of any antimuscarinic agent significantly reduced the degree of BH relative to HAL only treated animals. TRI in combination with HAL produced a dose-dependent reduction in BH. At the highest dose, their response rate was no longer statistically different from controls. TRI when given following chronic HAL pretreatment potentiated the behavioral response to APO. In complementary studies, we observed that chronic treatment with TRI reversed its acute potentiation of APO-induced stereotypic behavior. Vehicle pretreated animals given TRI demonstrated augmentation of APO stereotypy. In contrast, TRI pretreated animals manifested decreased responsiveness when challenged with TRI and APO.

The decrease in BH following the co-administration of antimuscarinic agents with HAL suggests that the cholinergic, as well as the DA systems participate in the expression of BH perhaps through supersensitization of muscarinic receptors.

309.13 EFFECTS OF PHENCYCLIDINE ON LOCOMOTOR ACTIVITY FOLLOWING INTRACEREBRAL INJECTIONS. H.D. Everist and A. Pert. Biological Psychiatry Branch, NIMH, Bethesda, MD 20205.

Phencyclidine (PCP) has been reported to have significant effects on locomotor behavior. Low doses appear to stimulate locomotor output while high doses result in an initial depression which is followed by excitation. Locomotor excitation induced by PCP may involve the dopamine (DA) system since DA receptor blockers are effective in attenuating this response. Little more, however, is known regarding the precise mechanisms or loci of action of PCP in producing alterations in locomotor behavior. The purpose of these studies was to ascertain the loci of action of PCP in brain in relation to locomotor output and to define its mechanism of action.

For the first study, rats were implanted with cannulae guides aimed for the lateral ventricle. PCP (5, 25, 75 n moles) was injected intraventricularly and locomotor activity was measured over the next three hours. All doses of PCP produced depression of locomotor activity during the first 30 minutes post injection. Only the two highest doses, however, enhanced locomotor activity during the next 30-45 minutes.

In the next series of studies, rats were implanted with cannulae guides aimed for a variety of brain structures. The animals were injected bilaterally with 12.5 n moles of PCP. Injections into the ventral thalamus, caudate nucleus and ventral tegmental area had no effect on locomotor output. Injections into the nucleus accumbens, on the other hand, produced an immediate and significant elevation in both horizontal and vertical components which seemed to persist over the course of an hour. This effect was reversed by pretreatment with 0.25 mg/kg haloperidol i.p., suggesting dopaminergic involvement. Injections into the periaqueductal gray matter and mesencephalic reticular formation had little effect on horizontal activity while significantly depressing the vertical component during the first 15-30 minutes. These findings suggest that PCP exerts its differential effects on locomotor behavior through different brain structures.

309.14 THE EFFECT OF DESIPRAMINE, MIANSERIN, AND YOHIMBINE ON ALPHA2-ADRENOCEPTOR FUNCTIONAL SENSITIVITY IN THE RAT. P. A. Seymour and R. G. Browne. Central Research Division, Pfizer, Inc., Groton, CT 06340.

Many investigators have examined the effects of antidepressants on alpha2-adrenoceptor sensitivity. However, the reported results have often been contradictory and inconclusive (Heal et al., Neuropharmacology, 22:983, 1983; Raiteri et al., Eur. J. Pharmacol., 91:141, 1983), which may be attributable to the use of different treatment and measurement variables among investigators. The present investigation examined the effects of two mechanistically distinct antidepressants, desipramine (DMI) and mianserin, on alpha2-adrenoceptor sensitivity, using clonidine-sedation as a measure of receptor functional sensitivity. Additional studies examined the effects of the alpha2-adrenoceptor antagonist, yohimbine, and the combined treatment of yohimbine with either DMI or mianserin on alpha2-adrenoceptor sensitivity. All treatments were administered for 1, 2, 4, 8, and 16 days. Twenty-four hours after the last drug administration, animals were injected with clonidine (.1 mg/kg), and exploratory locomotor activity (ambulation and rearing) was measured for six hours, in computer-monitored behavioral chambers. The data revealed that 1) DMI pretreatment induced rapid, dose-dependent and progressively increasing alpha2-adrenoceptor subsensitivity; 2) mianserin pretreatment induced rapid, non-dose-dependent alpha2-adrenoceptor supersensitivity; and 3) yohimbine pretreatment induced inconsistent, non-dose-dependent alpha2-adrenoceptor supersensitivity. It was also found that coadministration of yohimbine blocked the DMI-induced subsensitivity and potentiated the mianserin-induced supersensitivity of alpha2-adrenoceptors. In addition, DMI pretreatment significantly increased, while yohimbine and/or mianserin pretreatment significantly decreased, the exploratory activity of the vehicle-treated animals. Coadministration of yohimbine blocked the DMI-induced increase in activity. It was concluded that DMI and mianserin induced opposite effects on alpha2-adrenoceptor sensitivity, and, therefore, that the induction of alpha2-adrenoceptor subsensitivity is not a common mechanism of action of all antidepressants.

309.15 NORADRENERGIC PHARMACOLOGY OF THE BEHAVIORAL RESPONSE TO NOVELTY. *A. Garcia, *M. Varela, *M. Rosenthal and D.R. Britton (L.D. Partridge, sponsor) Departments of Physiology and Medicine, University of New Mexico School of Medicine, Albuquerque, N.M. 87131.

Previous studies have suggested a role for the central noradrenergic system in mediating animal behaviors which are seen as analogous to human anxiety. The nature of this role, if any, remains unclear due to differences in the animal models used and the methods of manipulating the level of central noradrenergic tone. Some studies with monkeys have suggested that the locus coeruleus noradrenergic system becomes overly active during states of anxiety and quiescent during non-anxious states (Redmond and Huang, 1979). On the other hand, other studies have shown with rats that destruction of the locus coeruleus produces no effect (Koob et al., in press) or an increase (Britton et al., in press) in an animal's "anxiety-related" behavior.

In the present study, we have investigated the effects of pharmacological manipulation of the central noradrenergic system on the stress-related behavior shown by rats in response to environmental novelty. Fasted animals were treated with either the alpha$_2$ receptor agonist, clonidine (CLON) or the alpha$_2$ receptor antagonist, yohimbine (YOH) or with saline. Thirty min. later the rats were introduced to a novel modified open field in the center of which a single food pellet was secured. During the 10 min. period of the test records were kept of the amount of rearing and grooming, the number of approaches to the food, amount eaten and the incidence of urination. YOH increased grooming at doses of 1.0 mg/Kg and at higher doses suppressed rearing. Clonidine increased urination and decreased rearing at all doses (50 - 200ug/Kg) tested. Food consumption was increased in animals treated with 50 ug/Kg CLON. These results suggest that although the central noradrenergic system may mediate some components of an animal's response to novelty, manipulation of that system with peripherally administered YOH or CLON does not mimic the effects of more commonly accepted anxiolytic or anxiogenic agents.

309.16 LATERALIZATION OF ATTENTION IN APOMORPHINE-TREATED RATS. M. Pisa and H. Szechtman. Departments of Neurosciences and Psychiatry, McMaster University, Hamilton, Ont., L8N 3Z5.

Apomorphine, a dopamine receptor agonist, induces a clear preference for direction of turning in many rats. This turning bias could reflect a purely motor asymmetry, i.e., a lateralized activation of motor commands for turning. However, it might also reflect a drug-induced lateralization of investigative responses. The latter hypothesis was tested by examining the response to edges of 30 male Sprague Dawley rats which were placed on a 180 x 180 cm wooden black table for 1 h after s.c. injections of 1.25 mg/kg of apomorphine.

Twenty-five rats showed either little attraction for the edges of the table or no clear preference for side of the body exposed to the edges. In contrast, 5 rats walked close to the edges most of their time, 3 rats constantly looping counterclockwise and 2 rats clockwise. To examine whether lateralized attention for edges controlled the direction of locomotion of these 5 rats, a 50 x 50 cm piece of the wooden surface was removed from the center of the table, leaving a square hole in it, and the rats were repeatedly placed close to the edges of the hole. All 5 rats walked along the edges and around the corners of the hole in a direction opposite that taken to loop at the periphery of the table. Thus, direction of locomotion was reversed, but side of the body exposed to the edges remained the same, indicating that the position of the edge controlled direction of turns in these rats. Lateralization of attention for the edges was further demonstrated in these 5 rats by repeatedly placing them on the table with an edge on either their spontaneously preferred side or the opposite side. After being released, all 5 rats immediately and persistently locomoted along the edge, if it was on their spontaneously preferred side. If the opposite side was exposed to the edge, however, the rats immediately turned 180°, thus exposing their preferred side to the edge, and then proceded to locomote along it. When placed on parts of the table removed from the edges, 2 of the 5 rats showed no clear turning bias, and 3 rats showed a bias for turning toward the same side as the side of the body that they usually exposed to the edges, a result suggesting that lateralization of investigative responses could contribute to a turning bias despite the absence of distinctive proximal stimuli.

The present findings reveal that apomorphine can promote a strong lateralization of investigative responses in some intact rats. (Supported by grants from the MRC and the NSERC of Canada. M.P. is an OMHF scholar and H.S. a MRC scholar).

309.17 APOMORPHINE INDUCES POSTURAL ASYMMETRIES INDEPENDENT OF ASYMMETRIES IN THE DIRECTION OF TURNING. H. Szechtman and M. Pisa. Departments of Neurosciences and Psychiatry, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

After injection of the dopamine receptor agonist, apomorphine, many animals exhibit an asymmetry in turning; i.e., they turn more in one direction than in the other. What behavioural processes yield this asymmetry? This presentation indicates that after injection of apomorphine, most rats exhibit an asymmetry in postural support, and that this asymmetry is often coupled with an asymmetry in turning. However, a postural bias is neither a sufficient nor a necessary explanation for the directional bias because some rats exhibit either an asymmetry in postural support without an asymmetry in the direction of turning or vice versa. Rats were injected s.c. with apomorphine (1.25 mg/kg) and placed on a large glass table for 1 min every 5 min. Their behaviour was videotaped. Postural support was measured indirectly by counting the frequency of two or more steps in succession of a hindleg while the other hindleg remained rooted during turning. Amount of turning of the pelvis was measured using the Eshkol-Wachman Movement Notation. Of 7 rats analyzed, one did not show any postural or directional asymmetries. Four rats showed both a significant postural bias ($p<.05$ or less, paired t-tests on frequencies of steps in succession of right and left hindlegs at 5,10,20,30 and 45 min after injection) and a significant turning bias ($p<.05$ or less). One rat had a significant postural asymmetry but not a directional bias while the remaining rat showed a significant directional bias but not a postural bias. Therefore, while in most rats the postural and directional asymmetries are coupled, they appear to be independent effects of apomorphine, a conclusion consistent with the lack of a significant association between these variables (Fisher exact test). Detailed analysis of the stepping patterns revealed that one basis for postural asymmetry is usage of different stepping strategies to turn in opposite directions. Thus, a postural asymmetry is neither necessary nor sufficient for a directional bias. As a bias in the direction of turning is associated with an interhemispheric imbalance in dopaminergic systems, the present findings suggest that such an imbalance may result in an asymmetry of other than motor processes (see Pisa & Szechtman, this volume, for an attentional hypothesis). (Supported by grants from NSERC and MRC. H. Szechtman is a MRC Scholar, M. Pisa is an OMHF Scholar.)

BEHAVIORAL PHARMACOLOGY II

310.1 LEARNED HELPLESSNESS INDUCED BY AN ACTIVE ANTAGONIST OF THE BRAIN BENZODIAZEPINE RECEPTOR. P.Skolnick, R.C. Drugan, S.F. Maier, S.M. Paul* and J.N. Crawley. (SPON: C.R. Creveling), Laboratory of Bioorganic Chemistry, NIADDKD, Bethesda, MD 20205, Department of Psychology, University of Colorado, Boulder, CO 80309, and Clinical Neuroscience Branch, NIMH, Bethesda, MD 20205.

Numerous behavioral and pharmacological studies have demonstrated that "learned helplessness" paradigms may represent an animal model of depression. The psychiatric literature suggests a close relationship between anxiety and depression. Recently, administration of derivatives of β-carboline-3-carboxylic acid have been shown to produce a behavioral, somatic, and endocrine syndrome characterized as "anxiety" or "fear" in both experimental animals and man. Blockade of these effects by the high affinity benzodiazepine receptor antagonist Ro15-1788 suggests that the β-carboline syndrome is mediated through central benzodiazepine receptors.

We have investigated the relationship between experimentally-induced "anxiety" and the development of learned helplessness by administering an anxiogenic benzodiazepine receptor "inverse" antagonist, N'-methyl-B-carboline-3-carboxylate (FG-7142) to rats twenty-four hours prior to exposure to a standard FR-2 shuttlebox escape acquisition task. Rats treated with FG-7142, 10 mg/kg i.p., failed to acquire an escape response twenty-four hours after treatment, in comparison with vehicle controls. However, the FG-7142 group performed the FR-1 control task as rapidly as the vehicle controls. Pretreatment of rats with the selective benzodiazepine receptor antagonist Ro15-1788, 20 mg/kg i.p., blocked the development of learned helplessness elicited by FG-7142. Thus, administration of an anxiogenic B-carboline appears to result in a behavioral effect similar to a session of inescapable shock. The involvement of the benzodiazepine-GABA-chloride ionophore receptor complex suggests that the protective effects of control over a stressor may be linked to central mechanisms mediating anxiety.

310.2 EFFECT OF INJECTION OF CHOLINE MUSTARD INTO MEDIAL SEPTAL AREA OF RAT BRAIN ON BIOCHEMICAL AND BEHAVIOURAL PARAMETERS. L.A.Myles*,M.Steingart*,R.J.Rylett and E.H. Colhoun*(SPON: R. Doucette) Dept. Pharmacology and Toxicology, University of Western Ontario, London, Ontario, N6A 5C1.

The suggestion has been made that injections of nitrogen mustard analogues of choline into certain brain areas of rat can produce cholinergic hypofunction. We report the effects of injection of varying concentrations of choline mustard aziridinium ion (ChM Az) into medial septum of rat on cholinergic markers and passive avoidance behaviour. Male Sprague-Dawley rats were given a single injection of ChM Az in doses of 0.3, 1, 2, 3, 5, 7, 10 or 22 nmoles in a 1 ul volume via a chronic cannula placed stereotaxically into the medial septal area. The rats were sacrificed 10 days post-injection and ChAT and choline transport activity were measured in cortex, hippocampus and striatum. A histological examination of paraffin sections from control and treated brains was carried out to assess tissue damage at the site of injection. Exploratory behaviour in an open-field test was performed on control and 1 and 22 nmole ChM Az-treated rats; short-term memory retention was measured by a passive avoidance task in control and 0.3 and 1 nmole ChM Az-treated animals.

Injection of 22 nmoles ChM Az into medial septum decreased ChAT activity by 68% and high-affinity choline transport by 76% in hippocampus. Choline transport was decreased by 37% in cortex but not in striatum; there was no reduction in ChAT activity in these brain areas. No significant changes in cholinergic markers were observed in these three brain areas in animals treated with 0.3 or 1 nmole ChM Az. Animals receiving 22 nmoles ChM Az showed decreased exploratory behaviour in the open-field test compared to saline controls. However, no significant difference was observed in the 1 nmole rats. Additionally, rats injected with 0.3 or 1 nmole ChM Az did not show consistent impairment when tested with a one-trial step-down passive avoidance task with no escape contingency. Histologically, large areas of non-specific necrosis were found at the site of injection in rats given doses above 3 nmoles. At lower doses, gliosis was observed in the area of the medial septum but necrosis was decreased. In summary, it would appear that a single acute injection of this compound does not produce a selective destruction of cholinergic cells at low doses. Investigation of other administration regimens may result in increased selectivity.

(Supported by the Ontario Mental Health Foundation)

310.3 EVALUATION OF THE ANXIOLYTIC POTENTIAL OF SELECTED 5-HT RECEPTOR ANTAGONISTS IN LABORATORY CONFLICT PROCEDURES. J.B. Patel and J.B. Malick. Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, DE 19897.

Earlier investigations into the mechanism of action of the anxiolytics have shown the importance of 5-HT pathways in their therapeutic action. Recently, agents that are selective 5-HT_2 receptor antagonists such as pirenperone (P) have been reported. In this study, a selected group of 5-HT antagonists were evaluated for anxiolytic potential in conflict models in animals. Pirenperone (MED = 0.4 - 0.8 mg/kg, i.p.) exhibited significant dose-dependent anticonflict activity in the shock-induced suppression of drinking (SSD) procedure in rats. Other 5-HT antagonists were found to be markedly less potent, as disinhibiting agents; for instance, cinanserin (C) demonstrated significant anticonflict effects only at 50.0 mg/kg, i.p. The anticonflict effects of these agents were not blocked by the benzodiazepine receptor antagonist, RO 15-1788. Preliminary data suggests that unlike the benzodiazepines which also exhibit activity in rat and monkey lever hold-down conflict tests, both (P) and (C) failed to produce significant increases in the number of shocks taken in these tests. Antagonism of 5-HTP induced head-twitching in mice was used to assess 5-HT antagonist activity. Thus, the benzodiazepines and the 5-HT antagonists exhibit a different spectrum of activity in conflict procedures; the implications of this difference will be discussed.

310.4 THE ACUTE BEHAVIORAL TOXICITY OF TABUN IN RATS. M. R. Landauer*, and J. A. Romano (SPON: J. Gibbons). US Army Chemical Research and Development Center, and US Army Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

A potent organophosphorus compound tabun (ethyl-N-dimethyl phosphoramidocyanidate) was evaluated for behavioral toxicity using a rotarod performance (RTR), spontaneous motor activity (SMA) and conditioned tests aversion (CTA) test, as well as the LD50 in 158 adult male rats. In Exp.1, the 24 hr LD50 was calculated using seven doses of tabun (N=8 per group) and found to be 240 µg/kg. In Exp. 2, motor coordination was evaluated by placing rats on an accelerating rotarod (0-45 rpm in 90 sec). Time spent on the rotarod in seconds was recorded as the dependent variable. Rats received four training trials and 30 min prior to the 90 sec test trial they received a s.c. injection of 100, 122, 144, 168 or 198 µg/kg tabun or saline (N=6-8/per group). Doses of 168 and 198 µg/kg produced performance decrements that differed significantly from saline control values. The ED50 was 171 µg/kg. In Exp. 3, locomotor activity was measured by placing an animal into an open-field (41 X 43 cm) and determining the number of photobeam interruptions in a 30 min test. All animals received three 30 min acclimation trials and were administered 100, 122, 144, 168, or 198 µg/kg tabun or saline vehicle prior to the fourth (test) trial (N=5/6 per group). Motor activity was significantly decreased from control values for the three highest dose groups and the ED50 was determined to be 154 µg/kg tabun. In Exp. 4, rats were acclimated to a daily 30 min period of water availability. When consumption had stabilized, they were given a 30 min access to a period of 0.2% saccharin (SAC) solution what was immediately followed by a s.c. injection of 100, 122, 144 or 168 µg/kg tabun or saline control vehicle. Three days later, the animals (N=6 per group) were given a two-bottle choice test (SAC vs. water) and the percent SAC consumed was calculated. Significant changes from saccharin preference were noted at the three highest doses and the ED50 was found to be 130 µg/kg. The ED50/LD50 ratio for the behavioral measures were thus 0.71, 0.64, and 0.54 for RTR, SMA, and CTA, respectively. Of the tests evaluated the CTA appeared to be the most sensitive in detecting the behavioral toxicity of tabun, a result found previously with the related compound, sarin (Landauer et al, Soc. Neurosci. Abstr., 1983,9,135).

310.5 FAMILIARITY, ANXIETY, AND ETHYL-BETA-CARBOLINE-3-CARBOXYLATE (A BENZODIAZEPINE ANTAGONIST). J.A. Wagner* and R.J. Katz. Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218 and Clinical CNS Research Group, Division of Pharmaceuticals, Ciba-Geigy Corp., Summit, NJ.

Ethyl-B-carboline-3-carboylate (B-CCE) is a potent antagonist and putative inverse agonist of benzodiazepine (BZ) receptors. In addition, B-CCE produces anxiety in a variety of behavioral tests. The present experiments explored changes in B-CCE-induced anxiety associated with changes in environmental familiarity. Two tests were used. A first test assessed the effects of B-CCE on the consumption of a novel food substance. Adult male Sprague-Dawley rats exposed to chocolate milk for 1 hr/day increased their consumption monotonically across days until maximal consumption was reached by day 10. Rats injected with B-CCE (0, 1, 5, 10 mg/kg; IP) on the fourth day of familiarity with chocolate milk decreased rather than increased consumption on the day of treatment in a dose-dependent fashion ($F(3,36)=4.65$, $p<.05$). Normal consumption returned on the following day. The B-CCE-induced decrease in consumption (1) was blocked by concomitant injection with Ro 15-1788 (10 mg/kg), a specific BZ antagonist ($F(3,20)=3.69$, $p<.05$), (2) was blocked by thorough familiarization with the food, and (3) differed from decreases due to sickness-inducing drugs. Results suggest that the decrease in consumption due to B-CCE is (1) at least partially mediated by BZ receptors, (2) specific to a neophobic motivational system, and (3) behaviorally different from effects of sickness-producing drugs. A second test was used to further explore the effects of familiarity. Rats, either familiar or unfamiliar with the apparatus, were conditioned with B-CCE (same dose range) in a place aversion procedure (Wagner & Katz, Neurosci. Lett., 43:333, 1983). Results indicate that, in comparison with vehicle, B-CCE produced a dose--dependent place aversion ($F(3,40)=12.7$, $p<.001$). However, the B-CCE-induced aversion was restricted to the rats familiar with the apparatus (familiarity, $F(1,40)=4.3$, $p<.05$; familiarity X dose interaction, $F(3,40)=3.5$, $p<.05$). Both sets of results suggest that anxiety induced by B-CCE can be modified by changes in environmental familiarity. These results imply that familiarity is a variable which needs to be controlled in future behavioral tests of putative anxiogenic compounds.

310.6 **EFFECT OF INTRACRANIAL DRUG INFUSIONS ON RESPONDING MAINTAINED UNDER A FIXED-INTERVAL SCHEDULE OF FOOD PRESENTATION.** **S.I. DWORKIN AND N.E. GOEDERS. Psychiatry Research Unit, Departments of Psychiatry and Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.**

With the exception of studies on the reinforcing properties of drugs, direct investigations of the involvement of different neurotransmitter systems or specific brain regions in behavioral effects of drugs have been limited. Neurotoxin lesion procedures have resulted with some success in determining neurobiological substrates of drug effects. However, lesions produce a relatively long term and/or permanent alteration in brain neurochemistry. Few researchers have investigated the effects of a drug administered directly into specific brain regions on schedule-controlled behavior with rats, perhaps because of limitations in technology. However, a new drug delivery system which allows administration of very small drug quantities during ongoing behavior has been developed (Bozarth and Wise, J. Neurol Method, 2:275, 1980). This system has been used to map different brain areas that are involved in the reinforcing properties of drugs. This study reports an investigation of the effects of intracranial injections on schedule-controlled behavior using the EMIT system. This methodology allows assessment of the involvement of different brain areas in the effects of drugs on schedule-controlled behavior. Male Fischer rats were prepared with intracranial microinjection cannulae and trained to respond on a fixed-interval 2-min schedule of food presentation. The effects of several drugs and neurohumors were investigated in 6 rats with bilateral cannulae implanted into the nucleus accumbens and 4 subjects with unilateral prefrontal cortex cannulae. Dose of morphine, dopamine and cocaine that are self-administered had no effect on fixed-interval responding. Furthermore, larger doses of these drugs as well as atropine did not alter responding. This study demonstrates that response-contingent incracranial drug infusions can have reinforcing effects in the absence of any effect of response-independent injections on fixed-interval performance. These data suggest that different brain regions may be involved in the reinforcing and rate-dependent effects of drugs. **Supported in part by USPHS Grant DA05252 (SID) and USPHS Grant MH-09222 (NEG).**

310.7 DIFFERENTIAL EFFECTS OF FORMAMIDINE PESTICIDES ON MULTIPLE FIXED-INTERVAL RESPONDING IN RATS. V.C. Moser* and R.C. MacPhail* (SPON: P.H. Ruppert) Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, N.C. 27711

Chlordimeform (CDM), amitraz, and formetanate (FMT) are members of the formamidine class of pesticides. To date, effects on operant behavior have been determined only for CDM. This experiment compared the effects of CDM, amitraz, and FMT on schedule-controlled responding. Nine male Long-Evans rats were trained during one-hour sessions to lever-press under a multiple fixed-interval (FI) 1-min:FI 5-min schedule of milk reinforcement. The rats were divided into three groups of three, with each group receiving each compound in a counterbalanced order. All compounds were given on Tues. and Fri. except during the amitraz determinations when, due to prolonged side effects, a week or more separated the high doses. All injections were administered i.p. 10-20 min pre-session. The doses were: CDM HCl, 0.3-20 mg/kg; FMT HCl, 0.03-0.75 mg/kg; and amitraz, 5-75 mg/kg (suspended in 5% emulphor and 5% ethanol). Under baseline conditions response rates were higher under the FI 1-min than under FI 5-min, and index of curvature (IOC) values (a measure of within-interval response patterning) were generally higher under the FI 5-min. All compounds produced dose-dependent decreases in response rate. CDM decreased FI 1-min response rates more than FI 5-min rates; a similar effect of amitraz was seen only at intermediate doses. FMT decreased responding to the same extent in both components. CDM produced pronounced changes in the pattern of responding in both components, with IOC decreased more under FI 5-min than under FI 1-min. Amitraz produced greater decreases in IOC under FI 5-min at intermediate doses, but equal decreases at higher doses. As is often seen with carbamate pesticides, FMT did not appreciably decrease IOC in either component. High doses of amitraz produced general signs of poor health that persisted for several days after high doses. In addition, a greater effect on response rates and IOC in both components was obtained when amitraz (75 mg/kg) was given more than 10 days following another dose compared to when it was given 7 days or less after another dose. The effects of these three formamidines on operant performance are not similar, and these differences may provide a tool with which to further study this class of compounds.

310.8 THE EFFECT OF PERGOLIDE AND BROMOCRIPTINE ON MOTOR PERFORMANCE AND REINFORCEMENT EFFICACY. G.M. Heyman* (SPON: R.T. Bartus). American Cyanamid Co., Medical Research Div. of Lederle Labs., Pearl River, NY 10965.

This study used a mathematical model to evaluate the effect of pergolide and bromocriptine, dopamine agonists, on reinforced responding in the rat. In each study the experimental sessions consisted of a series of five variable-interval reinforcement schedules in which lever presses were maintained by water reinforcement. For all subjects, 8 in each study, the relationship between response rate and reinforcement rate was described by the equation for a rectangular hyperbola: $B = kR/(R + R_e)$, where B is response rate, R is reinforcement rate, and k and R_e are estimated parameters. The rectangular hyperbola is the basic model in several research areas, (e.g., enzyme kinetics and receptor binding). In behavioral studies it is called the "matching law", and the parameters have the following meaning: k is equal to the asymptotic response rate and R_e is equal to the rate of reinforcement that maintains a one-half asymptotic response rate. Pergolide decreased the asymptotic response rate, k, and it also decreased the rate of reinforcement that maintained a one-half asymptotic response rate, R_e. In contrast, bromocriptine only affected the asymptotic response rate, k; it did not systematically change the reinforcement parameter, R_e.

On theoretical and empirical grounds, the asymptotic response rate, k, has been interpreted as an index for motoric components of reinforced responding, such as topography, and R_e has been interpreted as the index for reinforcement efficacy. Adopting these definitions, pergolide altered both motoric and reinforcing aspects of responding, whereas bromocriptine altered only the motoric factors. That bromocriptine selectively altered the motor parameter is in contrast with previous studies that used the matching law to evaluate drug effects. Amphetamine increased reinforcement efficacy and increased the response rate asymptote, whereas two dopamine receptor blockers, pimozide and chlorpromazine, decreased reinforcement efficacy and decreased the response rate asymptote. That is, these drugs affected both parameters of the hyperbola, and in each case the dose threshold for the reinforcement effect was lower than the dose threshold for the motor effect. Consequently, bromocriptine is the first drug to show a selective motor effect according to the matching law approach to behavioral pharmacology.

310.9 EFFECTS OF THE ETHYL ESTER OF β-CARBOLINE -3-CARBOXYLIC ACID ON NON-PUNISHED AND PUNISHED RESPONDING IN THE RHESUS MONKEY. Glowa, J.R., Crawley, J.N., Skolnick*, P. and Paul*, S.M.: Clinical Neuroscience Branch, National Institute of Mental Health and Laboratory of Bio-organic Chemistry, National Institute of Arthritis, Digestive, Diabetes and Kidney Diseases, Bethesda, MD 20205.

The effects of the ethyl ester of β-carboline-3-carboxylic acid (β-CCE) and diazepam (DZ) were compared on punished and non-punished schedule-controlled responding in adult, male rhesus monkeys. Both drugs were given intravenously, 10 min before responding was assessed. The monkeys were maintained at 85% of their free-feeding weight, and chaired during experiments. Lever-pressing was maintained under fixed-interval (FI) 2-min schedule of food-pellet presentation, responding was suppressed (punished) by scheduling each tenth response during the FI to produce a 3-5 mA foot shock. Punished and non-punished responding were studied under separate experimental conditions; three monkeys were studied under the punishment condition first and then under the non-punishment condition. Responding of two other monkeys was studied in the reverse order. Rates of responding under the punishment condition were always lower than those under the non-punishment condition. Cumulative doses of β-CCE decreased non-punished responding over the range of 0.01-1.0 mg/kg; the ED_{50} was approximately 0.03 mg/kg. Cumulative doses of β-CCE did not reliably decrease punished responding until doses of 0.3 mg/kg were obtained. In contrast, DZ increased punished responding at doses (1.0 mg/kg or greater) that did not increase, or increased less, non-punished responding. Mean blood pressure (BP) and heart rate (HR) increased as a function of increasing dose. Higher doses of both β-CCE and DZ decreased BP and HR. The results show that behavioral effects of β-CCE can be obtained with doses as low as 0.01 mg/kg, and suggest, that in primates, punished responding is less sensitive to the rate-decreasing effects of β-CCE than is non-punished responding.

310.10 EFFECTS OF PYRIDOSTIGMINE ON BEHAVIOR AND CHOLINESTERASE LEVELS IN CYNOMOLOGUS MACAQUES. J.H. McDonough, H.E. Modrow*, A. Kaminskis* and M.Z. Mays*. USAMRICD, Aberdeen Proving Ground, MD 21010.

As part of an ongoing series of studies of carbamate compounds the present work was designed to relate the behavioral effects of the quarternary carbamate pyridostigmine to the inhibition of blood cholinesterase (ChE) levels. Since carbamates produce dose related reductions in response rates on food motivated operant tasks, the dose-effect relationship of pyridostigmine was first determined on variable interval (VI) 60 sec performance for food pellet reinforcement. After behavioral testing was completed, blood ChE dose x time profiles were determined in the same subjects using similar pyridostigmine doses.

Nine adult male cynomologus monkeys were trained to lever press on a VI 60 sec schedule for food reinforcement. Sessions were 1 hr/day, 5 days/wk. After stable performance was attained each subject was tested with each dose of pyridostigmine Br. (vehicle, 0.10, 0.18, 0.32, 0.56 and 1.00 mg/kg, im.) according to a Latin Square design. Drug was given on only one test session/week. A dose by time ANOVA of response rates demonstrated only significant effects for dose, indicating that performance did not vary over the four 15 min portions of a drug test session. Performance was normal following pyridostigmine doses of 0.10, 0.18 and 0.32 mg/kg. Responding was significantly depressed only by the 0.56 mg/kg (55% of baseline) and the 1.00 mg/kg (4.6% of baseline) doses of pyridostigmine.

In the second phase the effects of 0.056, 0.10, 0.18 and 0.32 mg/kg, im. pyridostigmine on whole blood ChE levels was determined at 0, 5, 10, 20, 40, 80, 160 and 320 min after drug administration. These doses produced ChE levels of 59.9, 46.9, 37.3 and 21.5% of control values at the time of peak inhibition. At all doses maximal inhibition occurred at 40 min after injection. Muscle fasciculations were observed at doses as low as 0.18 mg/kg.

The results of this study demonstrate that pyridostigmine affects operant performance at dose levels that produce > 80% inhibition in whole blood ChE values. Since pyridostigmine is primarily peripherially acting, the observed performance decrements are best attributed to the motor and gastrointestinal effects of this anticholinesterase. Of perhaps greater interest is the finding that performance was not affected at doses that produced $\leq$ 80% ChE inhibition while previous work with centrally acting anticholinesterases indicates inhibition $\geq$ 50-60% is sufficient.

310.11 AMPHETAMINE CUE: ELICITATION BY INTRA-ACCUMBENS MICROINJECTION. E.B. Nielsen and J. Scheel-Krüger. Psychopharmacol. Research Lab., Sct. Hans Hospital, DK-4000 Roskilde, Denmark.

Previous research in our laboratories has demonstrated that both classical and atypical neuroleptics block the discriminative stimulus properties of 1 mg/kg d-amphetamine sulphate in the rat (two-lever, water-reinforcement, fixed ratio 32 operant task). Moreover, this cue was not mimiced by either apomorphine or lisuride. These findings may suggest that the amphetamine cue is mediated by mesolimbic dopamine (DA) systems which are distinct from those mediating stereotyped behavior induced by high doses of amphetamine and DA agonists. This hypothesis was examined by subjecting rats to 1) discriminate 1 mg/kg of amphetamine from saline; 2) stereotaxically implanting guide cannulas for bilateral microinjections into the nucleus accumbens (coordinates after histology A 9.4-9.8, L 1.1-1.6, DV -(1-1.4) according to König and Klippel, 1963); 3) ten days of discrimination re-training; 4) one intra-cerebral injection test session; 5) two discrimination training sessions; 6) a second intra-cerebral injection test session; 7) histology. The bilateral injection into the nucleus accumbens of 0.25, 1, and 5 µg of d-amphetamine produced 56% (N=6), 73% (N=3), and 93% (N=6) responses on the amphetamine appropiate response lever. The intra-accumbens effect of 1 µg of amphetamine was reduced dose-dependently to 50% (N=3) and 19% (N=3) by mixing 50 and 100 ng, respectively, of (-)-sulpiride with the amphetamine in the microinjection bolus. However, the stimulus effect of IP amphetamine (1 mg/kg; the training condition) was unaffected by sulpiride (50 and 100 ng) injected into the accumbens. These results indicate that stimulation of DA receptors in the accumbens produces the same discriminable effect as IP amphetamine but, that other (DA) neuronal systems may also be site(s) for perception of the global cue effect itself. This is currently under investigation in our laboratory.

310.12 CHOLECYSTOKININ (CCK) AND PHENYLPROPANOLAMINE (PPA) SHARE DISCRIMINATIVE STIMULUS PROPERTIES WITH AMPHETAMINE. J. Stafford and B. Hoebel. Dept. Psychology, Princeton Univ., Princeton, NJ 08544.

The drug discrimination paradigm is widely used to characterize drugs based on their discriminative stimulus properties, which reflect underlying mechanisms of action. Drugs with similar discriminative effects as assessed by generalization tests are thought to share neuronal mechanisms with the training drug (Appel, White & Kuhn, In Stimulus Properties of Drugs: Ten Years of Progress, 1978). Animals trained to discriminate amphetamine from saline were tested for generalization to cholecystokinin (CCK) and phenylpropanolamine (PPA). It has been suggested that some of the effects of PPA and CCK are dopamine-mediated. PPA at high doses has been shown to increase locomotor activity, induce stereotyped behavior, produce anorexia and cause ipsilateral rotation in rats with unilateral lesions of the substantia nigra (Zelger & Carlini, Neuropharmacol, 20:839, 1981). CCK has recently been found to co-exist in dopamine neurons (Hokfelt et al., Nature, 285:476, 1980) and to support self-injection in the nucleus accumbens (Hoebel & Aulisi, Soc. Neurosci. Abstr., 1984). Thus, it is of interest to assess these drugs for any tendency to generalize to amphetamine which is a well-known anorectic, locomotor stimulant and catecholamine agonist.

Six female Sprague-Dawley rats were trained to discriminate i.p. amphetamine sulfate (1.0 mg/kg) vs i.p. saline in a two-lever choice procedure in which one lever delivered food pellets following pre-session amphetamine injection, and the other lever following saline. Subjects required 20 to 30 daily training sessions to meet a criterion of 90% correct responses on the first trial for 10 consecutive sessions. Test doses of CCK (0.1 - 0.8 mg/kg) or PPA (5 - 50 mg/kg) were then substituted for amphetamine in extinction test trials. Both compounds produced dose-dependent generalization to the amphetamine cue. PPA (10 & 20 mg/kg) produced 100% responding on the lever appropriate for amphetamine; CCK (0.1 - 0.2 mg/kg) produced a maximum of 80% amphetamine-appropriate responding. These tendencies to generalize from amphetamine to PPA and CCK were partially antagonized by the DA blocker pimozide (0.5 mg/kg).

These results indicate that PPA and CCK share stimulus properties with amphetamine and that the generalization is largely DA-mediated. (Supported by USPHS grant MH-35740 and Squibb Inst. for Med. Res.)

310.13 BEHAVIORAL AND NEUROCHEMICAL CORRELATES OF PHARMACOLOGY INVOLVING THE 5-HT1 RECEPTOR. D.G.Spencer, Jr., T. Glaser*, T. Schuurman*, and J. Traber*. Neurobiology Dept., Troponwerke, Neurather Ring 1, 5000 Cologne 30, West Germany.

Receptors in the CNS that bind tritiated 5-HT with high affinity have been hypothesized to represent a subtype of serotonin receptors, termed 5-HT1. 8-Hydroxy-2-(di-n-propylamino)tetralin (PAT) was recently shown to be a rather specific ligand for the 5-HT1 receptor, perhaps even demonstrating selectivity for a group of 5-HT1 receptors found pre-synaptically (Gozlan, et al., Nature, 305:140-142, 1983). The CNS pharmacology of PAT and other 5-HT1 ligands was investigated behaviorally and neurochemically. The behavioral tests consisted of observation of the "serotonergic syndrome" (flat body posture, forepaw treading, etc.) and the effects of these drugs in rats trained to discriminate 5-methoxy-dimethyltryptamine (5-OMe-DMT) from saline, in a drug discrimination paradigm. The neurochemical tests consisted of effects on release of labelled 5-HT from rat cerebral brain slices and competition with labelled 5-HT in binding to rat hippocampal membranes.

In behavioral observation, both PAT and TVX R 1531 were potent in producing the serotonin syndrome. These two compounds also dose-dependently generalized to the 5-OMe-DMT stimulus. Both compounds decreased potassium-stimulated 5-HT release and potently competed with 5-HT binding. The actions of other serotonergic agonists and antagonists were also presented.

TVX Q 7821 is a putative anxiolytic that also competes potently with 5-HT for the hippocampal receptor. However, this compound does not decrease 5-HT release from cortical slices. At doses higher than those required for anxiolytic-like behavioral effects in animals, TVX Q 7821 also produces the serotonin syndrome and generalizes to the 5-OMe-DMT stimulus. These data support the notion that PAT and TVX R 1531 are pre-synaptic 5-HT1 agonists, but indicate that TVX Q 7821 may interact with this receptor in a different way.

310.14 INTRACRANIAL SELF-ADMINISTRATION OF DOPAMINE INTO THE NUCLEUS ACCUMBENS. G.F. GUERIN*, N.E. GOEDERS, S.I. DWORKIN and J.E. SMITH. (SPON: L. BETTINGER). Psychiatry Research Unit, Departments of Psychiatry and Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.

Dopaminergic innervations of the nucleus accumbens (NA) have been shown to be involved in both stimulant and opiate intravenous self-administration. 6-OHDA lesions of the NA decrease cocaine and amphetamine self-administration while increasing that of morphine. The mesolimbic dopaminergic system is also thought to participate in central reinforcement processes. A variety of drugs and endogenous substances have been shown to be self-administered into specific brain regions suggesting that the resultant changes in neuronal activity are reinforcing in those areas. If dopamine (DA) is involved in reinforcement processes, then its direct application into the brain may also initiate such neuronal activity. This experiment was designed to determine if rats would self-administer DA directly into the NA. Eight rats were implanted with unilateral injection cannulae into the NA and allowed to self-administer picomolar doses of DA on a fixed-interval 1-min schedule. Infusions were 100 nl in volume delivered over 5 sec and were paired with tone and light stimuli followed by a 1-sec timeout. Responses made before the end of the interval resulted in the brief presentation of the stimuli. Rats were sacrificed, brains removed and cannulae placements histologically verified. Rates of DA self-administration were significantly higher than vehicle. Moreover, it took several sessions for rates to decrease when DA was replaced with vehicle suggesting that its administration has potent reinforcing stimulus properties. These data indicate that DA is involved in the central mediation of reinforcment. It has been reported, however, that DA applied to the NA increases locomotor activity. The maintenance of responding under a fixed interval schedule suggests that the non-specific or motoric effects of DA did not confound the reinforcing properties of the neurotransmitter. Two-lever choice/reversal and receptor blockade studies are now in progress to further characterize this behavior. Although these data support the central dopamine theory of reinforcement, preliminary studies suggest that other brain areas and neurohumors are also involved in these processes. (Supported in part by USPHS Grant DA-01999 to JES, USPHS Grant MH-09222 to NEG, and USPHS Grant DA05252 to SID).

310.15 BLOCKADE OF NUCLEUS ACCUMBENS OPIATE RECEPTORS ATTENUATES THE REWARDING PROPERTIES OF INTRAVENOUS HEROIN SELF-ADMINISTRATION F.J. Vaccarino, F.E. Bloom and G.F. Koob. Scripps Clinic and Res. Fdn., La Jolla, CA 92037.

Evidence that cocaine and heroin are reinforcing drugs comes from studies showing that rats will self-administer these drugs intravenously (iv). While, current findings indicate that mesolimbic dopamine (DA) function is critical for the maintenance of iv cocaine self-administration, the neurochemical mechanisms underlying heroin self-administration are not yet clear. The present study attempts to further elucidate the neurochemical substrates of iv heroin self-administration by examining the effects of methyl naloxonium chloride (MN) (an opiate antagonist which does not readily cross the blood brain barrier) microinjections into either the ventral tegmental area (VTA) or nucleus accumbens (N.Acc.).

Rats with cannula implants aimed at either the VTA or N.Acc. were trained to self-administer heroin (0.06 mg/kg/injection) iv. Following stable responding each rat received microinjection of MN into either the N.Acc. or VTA ten minutes prior to self-administration tests. The following doses were tested: 0.0, 0.125, 0.25, 0.5 and 1.0 µg. MN was dissolved in saline and administered bilaterally in a 2 µl volume over 2 minutes. Doses were administered in ascending order with a minimum of three no pretreatment days separating drug tests. Following completion of MN testing N.Acc. rats were switched from heroin to cocaine reward. Following stable responding on cocaine (1.0 mg/kg/injection), these rats received intra-N.Acc. microinjections of 0.5 µg MN (optimal dose) ten minutes prior to the self-administration session.

The results demonstrate that intra-N.Acc. MN produces a dose-dependent attenuation of heroin reward as reflected by a dose-dependent increase in responding. Intra-VTA MN had no significant effect on i.v. heroin self-administration. Also, intra-N.Acc. microinjections of MN (0.5 µg) had no significant effect on cocaine self-administration. Taken together, these results suggest that opiate receptors in the nucleus accumbens are critical in the mediation of heroin reward. Furthermore, the findings that MN microinjections into the VTA did not influence heroin self-administration and that MN microinjections into the N.Acc. did not affect responding for cocaine are additional support for the hypothesis that separate neural systems mediate stimulant and opiate reward. This research was supported by grant DA 03665 from the National Institute on Drug Abuse.

CNS NEURONS II

311.1 SOME EFFECTS OF TONIC AFFERENT ACTIVITY ON INPUT FROM INDIVIDUAL SYNAPSES AS MODELED IN A CORTICAL PYRAMIDAL CELL OF KNOWN MORPHOLOGY. W.R. Holmes* and C.D. Woody (SPON: B. Swartz). Depts. of Biomathematics and Biobehavioral Sciences, UCLA, Los Angeles, CA. 90024.

Studies using the equivalent cylinder model for passive dendritic cables have shown that the electrotonic distance to most distal synapses is less than 2λ. However, tonic afferent activity is likely to affect estimates of electrotonic length, and in vivo and in vitro preparations used to study the same cell type may have significantly different levels of such activity. The aim of the present study was to examine the effect of different levels of tonic afferent activity on the spread of single synaptic inputs in a cortical pyramidal cell.

To do this a passive-membrane, continuous cable model of an HRP-injected cortical pyramidal cell serially reconstructed by means of photo-montages was used. The virtue of this particular model (cf. Holmes and Woody, Soc. Neurosci. Abstr., 1983) was that conductance input from single synapses and tonically activated distributions of synapses could be introduced while allowing the potential at all dendritic branch points and terminations to be monitored over time. Effects of uniform and non-uniform synaptic distributions on the spread of single synaptic inputs with peak conductance 2nS and reversal potential +0mV were compared for different combinations of background afferent activity in which a conductance of 107nS was distributed among conductance-increase synapses (6-30nS) and conductance-decrease synapses (101-77nS).

With uniform distributions of the two types of synapses, the amplitude of the EPSP seen at the soma due to a single synaptic input was reduced with increased levels of afferent activity. This might be explained in part by a reduced driving force caused by the less negative resting potential seen with tonic activity and in part by shunting. Non-uniform distributions of the two types of synapses produced non-uniform resting potentials with variations in the efficacy of synaptic input. When conductance-increase synapses were concentrated distally and conductance-decrease synapses proximally, the strength of a distal synaptic input at the soma was enhanced with low levels of afferent activity and reduced with high levels of afferent activity (compared to that seen with uniform distributions of the synapses).

(This research was supported in part by AFOSR and USPHS.)

311.2 **INACTIVATING OUTWARD CURRENTS VARY AMONG LOBSTER STOMATOGASTRIC NEURONS.** **Katherine Graubard and Daniel K. Hartline,** Univ. of Washington (Zoology), Seattle WA, and Univ. of Hawaii (Bekesy Lab), Honolulu HI.

Evidence of three outward current systems was found in voltage clamp of cells in the pyloric system of lobster stomatogastric ganglion. Clamps from rest to moderate (40 mV) depolarizations were characterized by large (in excess of 100 nA), rapidly decaying net outward current (sometimes exhibiting two rate constants of decay). The large transient outward current is tentatively identified as belonging to **A** current (Conner and Stevens 1971) because it is potentiated by -20 mv conditioning pulses, reduced by depolarizing conditioning, and is TEA-insensitive. This **A** current is more pronounced in some cells (e.g., PD) than others (e.g., LP). Another outward current component, present in both PD and LP cells, is much less dependent upon conditioning, is maintained during long steps, and is TEA-sensitive; it seems to accumulate inactivation when repeating 0.5 sec voltage pulses at 2 sec intervals. A small outward current remains after TEA and 4-AP and is accentuated by inward TTX-resistant currents; presumably this is a calcium-activated potassium current. The relative contribution of **A** current appears to be correlated with the functional role of the cell in the network. (Supported by NIH grants NS 15697 and NS 15314).

311.3 CURRENT FLOW THROUGH HIPPOCAMPAL SLICES. John G.R. Jefferys, Sobell Department of Neurophysiology, Institute of Neurology, Queen Square, London WC1N 3BG. U.K.

The resistive properties of brain tissue are significant both functionally, in ephaptic (field) interactions, which have recently proved to be important in synchronizing hippocampal discharges (Jefferys, J.Physiol. 319, 143-152, 1981; Jefferys & Haas, Nature 300, 448-450, 1982; Taylor & Dudek, Science 218, 810-2, 1982), and practically, in the technique of current source density analysis, which estimates membrane current from the spatial pattern of extracellular potential.

400um slices of rat hippocampus and adjacent tissue were trimmed with scissors to be as nearly rectangular as possible. Current was applied in the plane of the slice through sinterred Ag/AgCl electrodes. To minimize shunting, slices were maintained under warm, moist 0_2 - $C0_2$ mixture, on a thin film of artificial c.s.f. which flowed slowly through a strip of lens tissue on a glass platform. Current density was calculated from the applied current and the cross-sectional area of the slice measured from the micromanipulator scales using the recording electrode as a probe. Thus tissue resistivity could be estimated from the evoked voltage gradients, and yielded values of 3.6-6.0Ω.m perpendicular to the cell layers. These values resemble those previously obtained with cerebellum, though the hippocampal slice is less anisotropic (Nicholson & Freeman, J.Neurophysiol. 38, 456-368, 1975; Yedlin et al. Exp. Neurol. 43, 555-569, 1974).

Profiles taken at 10 or 20μm steps revealed abrupt increases (X1.5-X3.0) in estimated local resistivity at the pyramidal and granule cell body layers and at the hippocampal fissure. These local variations in resistivity may enhance ephaptic interactions; they also can cause spurious deviations in the estimated current source density (as seen with the purely extrinsic currents used here) and thus present a pitfall that should be considered in this kind of analysis.

Supported by the Thorn Fund.

311.4 MODELLING THE INFLUENCE OF NEURONAL GEOMETRY ON MEMBRANE POLARIZATION INDUCED BY APPLIED ELECTRIC FIELDS. D. Tranchina* and C. Nicholson (SPON: J. Gordon). Courant Institute & Dept. of Biology, New York Univ., New York 10012; Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York 10016.

Refinement of brain stimulation requires a better understanding of the coupling between neuronal morphology and extrinsic electric fields. We present a method for computing the membrane potential in various parts of an idealized neuron subjected to an electric field. The model neuron consisted of a soma, a myelinated axon, and a dendritic tree that branched symmetrically. The diameters of the branches conformed to the 3/2 power rule of Rall, and the spatial branching pattern could be varied. The tissue encompassing the neuron was assumed to be a homogeneous conductive medium, and the field applied to it was not disturbed by the presence of the neuron. This allowed us to specify the external potential on the surface of the neuron. As a consequence of the model adopted, analytic solutions for the membrane potential at the soma and at the nodes of Ranvier could be derived. The problem was divided into two independent parts: an equivalent finite cylinder representation of the dendritic tree and a semi-infinite axon. Internal and external potentials in the equivalent cylinder problem corresponded to average potentials at a given electrotonic distance from the soma. The solutions to these problems were combined by satisfying conditions of voltage continuity and current conservation at the soma. We studied both constant applied fields and those generated by a monopolar point source. In the former case we showed that the polarization at the soma exceeded that in the axon where potential change diminished exponentially with node distance from soma. With point sources, results were dependent on the source position. This method can be applied to determine the relative susceptibility of different neuronal geometries to various applied fields. It is particularly relevant to neurons with oriented dendrites, such as elements of the cerebellum. Supported by USPHS grant NS18287 from NINCDS.

311.5 EFFECT OF APPLIED SINUSOIDAL ELECTRIC FIELD ON IDENTIFIED TURTLE CEREBELLAR NEURONS. C. Y. Chan* and C. Nicholson. Dept. Physiol. & Biophys. New York Univ. Med. Ctr., New York, NY 10016.

The interaction between electric fields and neurons is of importance both in understanding basic physiology and in applications involving electrical prosthesis. We determined how imposed low frequency electric fields modulate neuronal activities in isolated turtle cerebellum. The cerebellum was removed and supported horizontally on a nylon mesh across a hole in the partition of a recording chamber between two large horizontal Ag/AgCl plate electrodes. The whole assembly was bathed in oxygenated Ringer solution. Spontaneous single unit spikes or those evoked by a bipolar local surface electrode (LOC) positioned on the dorsal brain surface were recorded extracellularly using glass microelectrodes filled with NaCl or KCl with 3% HRP. Sinusoidal current (0.5-4 mA, 0.05-2 Hz) from an isolated current source was passed between the plate electrodes. Unit firing patterns in the presence and absence of the imposed current were discriminated and compared using a computer or a chart recorder to determine the extent of modulation by the current. The neuron was then impaled and filled with HRP. The induced electric field in the tissue was measured in each cerebellum and was found to be constant for a given current. Most neurons showed both spontaneous and LOC-evoked activities. Some were silent but responded to LOC stimulation, or were spontaneously active but not stimulated by the LOC. The cells recorded from were identified by electrophysiological criteria and anatomically after Co^{2+}-DAB histological treatment. Among identified neurons, the activities of Purkinje cells and deep molecular layer stellate cells were modulated while those of shallow interneurons having dendrites parallel to the surface were unmodulated. All modulated units responded to the field of 22 mv/cm or more. The neuronal modulation patterns suggested that current flow oriented from distal dendritic pole towards the soma accelerated firing while current flow in the opposite direction inhibited it, consistent with preferential activation by depolarization of the soma or adjacent proximal processes. Supported by USPHS Grant NS18287 from NINCDS.

311.6 BEHAVIOR OF THALAMIC CELLS IN THE ABSENCE OF RETICULARIS INPUT: EVOKED ACTIVITIES. L. Domich*, C. Mulle*, M. Steriade and M. Deschênes. Dépt. de Physiologie, Fac. de Médecine, Université Laval, Québec, Canada, G1K 7P4.

During synchronized sleep in chronically implanted animals or in barbiturized preparations, cortical stimulation triggers in most thalamic neurons spindle-like sequences of membrane potential oscillations. In a companion communication, we report that relay neurons disconnected from the reticularis thalami (RE) nucleus no longer display spontaneous spindle oscillations. In order to check if cortical stimulation could trigger such oscillations in relay neurons in the absence of RE input, the RE nucleus was destroyed by local injections of kainic acid. On the other hand, we have recently reported that, at variance with most thalamic nuclei, the anterior nuclear complex does not receive a RE input (Hada et al., Neurosci. Abstr., 9: 1213,1983). Recordings were then performed in the anterior ventral and anterior medial nuclei in order to see if these cells display spindle oscillations. Selective destruction of RE cells was performed in cats by a single injection of 0.1μl of kainic acid (1% of H_2O) in the internal capsule. In these preparations under barbiturate anesthesia, thalamic relay neurons of the ventrolateral nuclues could be backfired by motor cortex stimulation but in no instance did such stimulation induce rhythmic activities as observed in normal cats. Instead of the usual sequence of repetitive long-lasting hyperpolarizations, cortical stimuli were followed by a graded depolarization and a subsequent slowly-decaying hyperpolarization. When recordings were performed in the anterior nuclear group of non-lesioned barbiturized cats, spontaneous spindles were absent and they could not be triggered by stimulation of the parasplenial cortex. Nonetheless, anterior thalamic cells appeared to possess the same intrinsic membrane properties as found in relay neurons of other thalamic nuclei. Taken together, these results show that the interconnections between RE neurons and thalamic relay cells are essential for the genesis of the 7-14 Hz spindle oscillations. Supported by MRC grants MT-3689 and MT-5877.

311.7 BEHAVIOR OF THALAMIC CELLS IN THE ABSENCE OF RETICULARIS INPUT: SPONTANEOUS ACTIVITIES. C. Mulle*, L. Domich*, M. Deschênes and M. Steriade. Dépt. de Physiologie, Fac. de Médecine, Université Laval, Québec, Canada, G1K7P4.

The role of reticularis thalami (RE) neurons in the genesis of thalamic spindles was investigated in chronically implanted cats and in anesthetized preparations. In relay neurons of the lateral thalamus, spindle sequences were characterized by rhythmic hyperpolarizations of about 150 msec interrupted by high-frequency burst discharges. In RE neurons spindles appeared as sequences of rhythmic depolarizations with spike barrages at interval of about 150 msec. After transections that deprived relay neurons from their RE input, spindling was abolished in thalamic relay cells. In chronic cats or in cats anesthetized with ketamine, RE-deprived relay neurons displayed a peculiar rhythmic activity characterized by burst discharges recurring at a frequency of 1 to 2 Hz. In deeply barbiturated animals no such rhythm was recorded following the transection. Rhythmic burst discharges observed under ketamine were blocked by i.v. injections of bicuculline suggesting that IPSPs were at the origin of bursting activity. When recorded intracellularly the membrane potential of RE-deprived thalamic cells displayed numerous, short-lasting (≈10-15 msec) IPSPs, whose amplitude depended upon the anesthetic used: 4-8 mV under ketamine, 1-2 mV under pentobarbital. No rhythmic pattern could be detected in the occurence of IPSPs. Current pulses injections revealed that the kinetics of deinactivation of the Ca conductance underlying burst discharges was such that temporal integration of short-lasting hyperpolarizations could be carried out by thalamic cells. Rhythmic burst discharges in RE-deprived relay cells could thus result from non-rhythmic, subthreshold hyperpolarizations provided that the latter occur with a minimal temporal density, duration and amplitude. Supported by MRC grants MT-5877 and MT-3689.

311.8 REGIONAL CALCIUM ENTRY IN NEURONS OF THE GIANT BARNACLE DETECTED WITH ARSENAZO III. W.N. Ross, L.A. Lewenstein and N. Stockbridge*, Dept. of Physiology, New York Medical College, Valhalla, New York 10595.

Absorbance changes of the dye Arsenazo III were measured with an array of photodiodes to detect variations in the magnitude and time course of intracellular calcium changes among different regions of neurons in the supraesophageal ganglion of Balanus nubilus. After the iontophoresis of Arsenazo III into the cell, absorbance changes at 610-670 nm were monitored at each position simultaneously in response to depolarizing stimuli. Spectral measurements over the soma, axon, dendrites, and presynaptic regions confirmed that the changes were due to calcium concentration increases. Anatomical correlations to the optical signals were made by the injection of Lucifer Yellow and subsequent examination with fluorescence optics.

In many neurons action potentials caused an absorbance increase over all parts of the cell indicating that there were voltage dependent calcium channels in the soma, axon, and dendritic processes. When the action potentials were increased in width by the substitution of 50 mM TEA for Na in the saline the absorbance changes recorded over the fine dendritic processes saturated after several hundred milliseconds.

The decay of the absorbance increase was very sensitive to the concentration of injected Arsenazo III, increasing from about 1 sec to 15 sec as the dye concentration increased from 0.3 mM to 2 mM. At all dye concentrations the time constant was faster at the edges of the soma than at the center of the cell.

The wide distribution of voltage dependent calcium channels over the surface of these neurons also makes the absorbance changes a useful tool for examining the propagation of electrical potentials. For example, depolarizing pulses in saline containing TTX results in absorbance changes only over the somatic region of some cells, while in another cell (which has been demonstrated to support a propagating calcium spike in TTX) absorbance changes are found all over the cell. In other experiments an antidromic action potential fails to invade the soma when hyperpolarizing current is passed into the cell body. Absorbance signals, in this case, are found over the axon up to the region in the ganglion from where most of the processes branch, suggesting that this is the failure point.

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311.9 INACTIVATION OF Ca^{2+}-DEPENDENT K^+ CONDUCTANCE DURING REPETITIVE INTRACELLULAR STIMULATION IN HIPPOCAMPAL NEURONS. T.A. Pitler* and P.W. Landfield (SPON: T. Troost). Dept. of Physiol. & Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103

The Ca^{2+}-mediated inactivation of inward Ca^{2+} currents and Ca^{2+}-dependent K^+ conductance has been previously described for invertebrate neurons (cf. Eckert and Tillotson, J. Physiol., 1981; Eckert and Ewald, Science, 1982). However, it is not yet clear whether Ca^{2+}-mediated inactivation of Ca^{2+}-dependent processes also occurs in central vertebrate neurons.

Hippocampal frequency potentiation of the EPSP, which is very likely a Ca^{2+}-dependent process, gradually declines during repetitive synaptic stimulation. This raises the possibility that inactivation of Ca^{2+}-dependent processes might also occur in hippocampus. To investigate this, we studied a more clearly established Ca^{2+}-dependent process: The prolonged K^+ conductance (500-1000 ms) that follows depolarization in hippocampal neurons (Hotson and Prince, J. Neurophysiol., 1980; Alger and Nicoll, Science, 1980; Schwartzkroin and Stafstrom, ibid, 1980).

Trains of repetitive (10 Hz) intracellular depolarizing current pulses (40 ms), above threshold for spike initiation, were administered for 4-5 min to CA1 cells in hippocampal slices. Input resistance was assessed at several intervals with hyperpolarizing pulses. The depolarizing intracellular stimulation elicited a major increase in input conductance, which was found to be due to a Ca^{2+}-dependent K^+ conductance.

A clear decline of this stimulation-induced K^+ conductance increase was seen during the train; the decline began after 30-60 sec and was generally pronounced by 4-5 min of 10 Hz depolarizing stimulation.

Since a similar timecourse of inactivation is found for frequency potentiation of the EPSP, these studies indicate that Ca^{2+}-dependent processes may generally exhibit inactivation in hippocampal neurons during repetitive stimulation. However, the observation that inactivation does not begin for 30-60 sec of stimulation suggests either that residual Ca^{2+} may continue to gradually accumulate intracellularly, or that the Ca^{2+}-buffering capacity of the cell may decrease during prolonged stimulation.

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311.10 MODULATION OF THE FIRING RATE OF APLYSIA NEURONS BY SINUSOIDAL ELECTRIC FIELDS AND INTRACELLULAR CURRENTS. A.R. Sheppard, M.B. Sagan*, S.M. Bawin and W.R. Adey. J.L. Pettis Hospital and Loma Linda University, Loma Linda, CA 92357.

Field effects on neurons are implicated in synchronization of cells surrounding the goldfish M-cell and in single unit or population response of hippocampal CA1 neurons. In some models, field effects appear related to local coupling of neighboring cells, but our observations of field effects at levels of 1 to 50 mV/cm in Aplysia or rat hippocampus suggest a physiological role for fields distributed throughout the tissue.

We studied pacemaker cells of the Aplysia abdominal ganglion with either injected sinusoidal currents (ICs, approx. 0.2 to 2 nA amplitude) or extracellular sinusoidal fields (EFs, 3.3 or 6.7 mV/cm amplitude, produced by currents in the saline bath). A spherical cell model (that ignores neurite contributions) indicates field-induced somatic transmembrane current densities 70 fold weaker than those produced by a 0.2 nA soma-injected current. Although very different in their electrical coupling to the cell, both ICs and EFs can produce similar changes in pacemaker firing: both can drive cells near their natural firing rates (NFRs), although the EFs never lock cells to the same extent, and cells seldom stay phase locked to the EFs for periods > 10 s. Most commonly, EFs had a probabilistic influence on the phase angle, Φ, measured between the waveform and cell firing. (Φ defined as zero for a spike at the positive-going zero-crossing of the waveform.) At high ICs (2 nA), with frequencies near NFRs, Φ can be << 90 deg, and multiple spikes may occur for each depolarizing portion of the current. Weaker ICs tend to lock the cell firing between 90 and 180 deg. EFs may also lock cell firing between 90 and 180 deg, but with greater variability. In cases where locking does not occur, EFs nevertheless have a statistical influence: firing occurs more often at a fixed Φ, which varies from cell to cell (±180 deg). This suggests that the excitation process involves a summation of currents over the entire neuron, not uniquely related to field polarity in the medium.

(sponsored by Department of Energy and Southern California Edison Company)

311.11 GLUTAMATE MICROAPPLICATION AS A PHYSIOLOGICAL METHOD TO STUDY LOCAL NEURONAL CIRCUITS IN HIPPOCAMPAL SLICES.
E. P. Christian* and F. E. Dudek. Dept. of Physiology, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Local neuronal circuits are thought to play an important role in normal information processing and in pathophysiological processes, such as epilepsy. Nonetheless, the spatial arrangement of local circuits is unknown because of a lack of practical methodologies for exploring this issue. We therefore tested the technique of local application of glutamate (Glu) to rat hippocampal slices while recording postsynaptic potentials (PSPs) intracellularly from pyramidal cells as a potential probe for examining local circuits without stimulating axons.

Hippocampal slices were prepared using standard procedures. The slices were constantly perfused during the experiments with a standard mammalian medium, although K^+ ranged from 3-6 mM and Ca^{2+} from 2.4-4.8 mM to vary the level of background PSPs. In preliminary experiments, action potentials were recorded extracellularly from dentate granule cells while microdrops (10-40 µm dia.) of Glu (5-50 mM) were applied from glass micropipettes onto either the granule cell body layer or nearby areas containing the mossy fiber axons from these cells. Glutamate application to cell bodies within 100 µm of the recording site, but not to mossy fibers, increased spike frequency for 2-8 sec in a concentration-dependent manner. When CA3 pyramidal cells were recorded intracellularly, Glu microapplication to granule cell bodies but not mossy fibers produced an increased frequency of PSPs ($\geq$ 3 mV) lasting 1-8 sec. These data support the notion that microdrops of Glu in the concentration range studied selectively activate cell bodies but not axonal projections.

Glutamate was also applied directly to the CA3 cell body region at various distances from intracellularly recorded CA3 cells. Application within approximately 300 µm of the recording electrode resulted in an increased frequency of PSPs for 1-2 sec, followed by a slower depolarization-repolarization sequence. These increases in PSP frequency may be the result of Glu activation of local synaptic circuits in the CA3 area. Although the possibility that Glu may have activated presynaptic terminals cannot presently be ruled out, this technique deserves further consideration as a potential method for studying the spatial characteristics of local excitatory and inhibitory circuits in hippocampus and other brain structures.

Supported by NIH grant NS 16683.

311.12 VOLTAGE-SENSITIVE DYES SIGNAL SYNAPTIC POTENTIALS IN GOLDFISH OPTIC TECTUM IN VITRO. P. B. Manis* and J. A. Freeman. Dept. of Anatomy, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232.

The ability to examine transmembrane potentials using voltage-sensitive dyes is a new and potentially powerful tool for the study of functional synaptic organization. We report here that certain styryl dyes can signal membrane potential changes in the goldfish optic tectum.

Thick slices of the optic tectum were cut on a tissue chopper and maintained in oxygenated Ringers. Electrical stimulation of the optic tract produced extracellular field potentials similar to those seen in vivo, with an additional component at 20 msec latency ("L" wave). Application of the styryl dyes RH414, RH246, or RH355 immediately and irreversibly suppressed the L potential but only transiently affected the presumed monosynaptic potentials.

Changes in dye-induced fluorescence ($3x10^{-3}$ $\Delta F/F$) were observed following optic tract stimulation. The dye response consisted of two components: a 10 msec half-width depolarization with a 2-4 msec latency, and a long duration (>1sec) depolarization which began during the first response. The second portion of the response was about half the amplitude of initial depolarization. Superfusion with 0.2mMCa-5mMMg Ringers reversibly suppressed both components, indicating a post-synaptic origin. Interestingly, no fluorescence changes were associated with the presynaptic fiber volley or terminal potential, even when the objective was carefully focussed on the fasicles of optic fibers. All dyes tested (RH414, RH246, RH355) gave similar results. No optical changes occured in the absence of dye.

The first portion of the dye response probably reflects monosynaptic depolarization of tectal neurons. The time course and latency is similar to that of published intracellular recordings. The long duration of the second component is however at odds with published data. We are currently investigating the possibility that it is glial in origin. The lack of an observable presynaptic component may be explained by failure of the dye to access the optic nerve axon membrane because of ensheathing glial cells. Changing the focal level of the objectives affected both the rise time and latency of the dye responses. Therefore, it is likely that optical sectioning techniques will improve the spatial (and hence temporal) resolution of voltage-sensitive dye recordings. We thank Prof. A. Grinvald for his generous gift of the dyes used in this study. (Supported by EY-01777 to JAF and NS07377 to PBM).

311.13 GIANT FIBERS AND PECTORAL FIN ADDUCTOR MOTONEURONS IN THE HATCHETFISH, E. Gilat*, D.H. Hall, and M.V.L. Bennett, Dept. of Neuroscience, Albert Einstein Coll. of Med. Bronx, N.Y. 10461.

In the hatchetfish Gasteropelecus a major component of the Mauthner fiber (MF) mediated escape reflex involves bilateral activity of the pectoral fin adductor muscles. The MFs excite giant fibers (GFs) via chemical synapses and GFs in turn excite the pectoral fin motoneurons (MNs) (Auerbach, A.A. and Bennett, M.V.L., J. Gen. Physiol., 53:183 1969). Synapses from GFs to MNs are electrotonic and rectify so as to facilitate spread of depolarization from GFs to MNs. In the present study Lucifer Yellow CH (Sigma, 4% in 0.1M LiCL) was injected iontophoretically into GFs and MNs were studied by electron microscopy (EM). As previously shown, GFs originate from cell bodies about 25 µM in diameter in the lateral wall of the fourth ventricle. Each axon crosses the near MF from which it receives a single synapse and bifurcates to form a caudal and a rostral branch, which run longitudinally and receive several synapses from the other MF. The rostral branch continues anteriorly to synapse on a pool of neurons that presumably innervate cephalic musculature. The caudal branch of the giant fiber goes posteriorly to synapse on MNs innervating adductor muscle of the pectoral fin on that side. Most caudal branches also send a thin secondary branch back across the midline to the opposite adductor muscle MNs (ipsilateral to the GF soma). Preliminary EM study shows typical gap junctions (GJs) between MNs and large myelinated fibers. We are examining preparations with HRP injected GFs to determine whether the GJs couple GFs and MNs. No asymmetry of GJs that would account for rectification has been seen. Dye passage from injected GFs to postsynaptic MNs was not observed but may have been obscured by autofluorescence. The number of pectoral fin adductor MNs in the 1st spinal segment on each side was found to be 26.5±3 (n=4) in Nissl stained preparations while the number of autofluorescent MNs in fixed but unstained preparations was 24.2±6.9 (n=8). The autofluorescence emitted by the MNs is nonuniform and may arise in perinuclear aggregates of lysosomes seen by EM.

311.14 INTEGRATIVE MECHANISMS CONTROLLING DIRECTIONAL SENSITIVITY OF AN IDENTIFIED SENSORY INTERNEURON G.A.Jacobs, J.P.Miller & R.K.Murphey, Dept. of Biology, SUNY Albany, Albany N.Y. 12222 & Dept. of Zoology, UCB, Berkeley, Ca. 94720

We are studying how the structure of an identified interneuron in the cricket influences the integration of complex synaptic inputs. This neuron, which receives input from afferents associated with wind sensitive filiform hairs on the cerci of the animal, exhibits an overall directional sensitivity to wind stimuli. The cell has three distinct dendritic branches, and we have used two complementary techniques to demonstrate that each dendrite exhibits its own directional sensitivity. The techniques involved 1)selective activation of small patches of hairs known to overlap with individual dendrites and 2)selective inactivation of individual dendrites with a laser microbeam. Polysynaptic inhibition is known to contribute to the directional sensitivity of this neuron, and we have identified a class of afferents that activates this inhibitory pathway. Through selective laser ablations at different positions on the neuron the location of inhibitory inputs onto the cell has been determined. Similar ablation experiments revealed the probable location of the spike initiating zone. We now have a description of the location of excitatory and inhibitory inputs onto this neuron and have assessed their relative efficacies with respect to spike initation. This information has been used to provide a quantitative explanation for the relationship between synaptic input and the directionally selective spiking output of this neuron.

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NO ABSTRACT

313 WORKSHOP. THE SUBFORNICAL ORGAN AS A MODEL OF NEUROHUMORAL INTEGRATION. P.M. Gross, SUNY at Stony Brook (Chairman); H.D. Dellmann*, Iowa State Univ.; A.K. Johnson, Univ. of Iowa; R.W. Lind, Salk Inst.; M.I. Phillips, Univ. of Florida; L.P. Renaud, Montreal Gen. Hosp.; J.B. Simpson, Univ. of Washington; J.Y. Summy-Long, Penn. State Univ.

The numerous recent investigations on the subfornical organ (SFO) herald its emergence as a model structure of neuroendocrine regulation. The SFO derives embryologically from telencephalic ependyma at the convergence of the lamina terminalis and tela choroidea. It is strategically located for neuroendocrine activity as a midline tubercle protruding into the third ventricle at the interventricular foramina. Having highly specialized morphological features for detection of both humoral and neural signals, the SFO is a major brain target for the principal hormone of thirst, angiotensin II. An increase in vasopressin secretion upon SFO stimulation suggests its role within neuronal circuitry influencing the pituitary neural lobe. The organization of neural substrates and central integration of stimuli acting at the SFO are the highlighted topics of this workshop. The program is divided into halves devoted to 1) the underlying neuroanatomical and neurochemical bases for SFO function, and 2) responses of the SFO to stimuli of dehydration.

D. Dellmann will summarize morphological characteristics of the SFO, including its capillary bed, neural constituents, and ependymal cells. The anatomical connectivity of the SFO with other cerebral structures involved in thirst and fluid balance will be the discussion of W. Lind. Complementary to this analysis are the studies by L. Renaud who will discuss electrophysiologically-derived maps of SFO efferent projections. K. Johnson will describe the significance of these connections with relation to catecholaminergic interactions with structures along the lamina terminalis.

Enzymatic, metabolic, and neuroendocrine comparisons of the SFO with the hypothalamoneurohypophyseal system constitute the presentation by J. Summy-Long. P. Gross will summarize evidence showing elevated glucose utilization in the SFO in different rat models of thirst. The origin and localization of central interactions between peripheral and brain angiotensin, and their potential influence on the SFO and thirst responses, will be discussed by I. Phillips. The formal presentations of the workshop will conclude with J. Simpson's review of SFO contributions to physiological mechanisms governing body fluid balance.

DEVELOPMENT AND PLASTICITY: GENICULO-CORTICAL PATHWAYS

314.1 POSTNATAL DEVELOPMENT OF RECEPTIVE FIELD SURROUND MECHANISM(S) IN THE KITTEN DORSAL LATERAL GENICULATE NUCLEUS. J.S. Tootle* and M.J. Friedlander. Dept. of Physiology & Biophysics, Univ. of Alabama in Birmingham, Al 35294

Cells in the kitten dorsal lateral geniculate nucleus (LGN_d) have been reported to have large receptive field centers. This may be in part due to an immature receptive field surround mechanism(s). We have begun a quantitative study of the development of this mechanism (both the surround's antagonism of the center response and the surround's excitatory response) in the A-laminae of the 26-35 day-old kitten's LGN_d. Physiological maintenance, receptive field classification and intracellular filling with horseradish peroxidase were as previously reported.[1] In addition, intra-arterial blood pressure was continuously monitored and kept at a mean pressure >80 mmHg and <120 mmHg. Strength of surround antagonism was assessed with the area-response function technique and was quantified as percent increase in the area of a spot which reduced the cells response to half its maximal value. The maturity of the excitatory receptive field surround was assessed by 1) noting the percentage of cells which gave an excitatory surround response to annuli flashed in the receptive field surround, and 2) measuring the contrast threshold of the excitatory surround response while a standardized adapting spot was positioned in the receptive field center. Our sample to date includes 73 LGN_d cells (kittens: n=36, adult control animals: n=37). The strength of surround antagonism in most kitten cells was comparable to that measured in adult controls. No surround antagonism was evident however, in the response of two of the kitten cells, even with very large spot sizes. Fewer kitten cells were excited by annuli in the receptive field surround than were cells in the adult (18% vs. 81%). When the center mechanism was adapted, more cells (88% vs. 100% in kittens and adults, respectively) had excitatory surrounds. Threshold measurements showed that the excitatory surround mechanism is more sensitive to contrast modulation in the adult animals than in the kittens. We are currently relating the development of the cells' surround mechanism(s) to their morphological maturity.

(Supported by NIH grants EY03805, EY05714 and the Alfred P. Sloan Foundation).

[1]Friedlander, M.J. Nature 300, 180-183, 1982.

314.2 POSTNATAL DEVELOPMENT OF GENICULOCORTICAL Y-AXON TERMINAL ARBORIZATIONS IN AREA 18 OF THE CAT. M.J. Friedlander, K.A.C. Martin* and J.S. Tootle* (SPON: C.W. Oyster). Dept. of Physiology and Biophysics, Univ. of Alabama in Birmingham, Birmingham, AL 35294 and Dept. of Exp. Psychology, Oxford Univ., U.K.

The geniculocortical pathway of the cat undergoes a period of postnatal maturation. Cells of the dorsal lateral geniculate nucleus (LGN_d) are maturing structurally and functionally for at least 6 weeks postnatally.[1,2] Ocular dominance columns in visual cortex (which represent the distribution of geniculocortical axon terminals) are also maturing and partitioning during this period.[3] We have begun to study the morphological development of geniculocortical axons (particularly Y-axons which project to area 18). Axons in the optic radiations were electrophysiologically characterized on a battery of tests (including linearity of spatial summation). The axons were then filled with horseradish peroxidase (HRP) which labeled their terminal arborization in visual cortex. Our sample to date includes 10 Y-axons that project to area 18 (6 axons from 4-5 week old kittens and 4 axons from adult control animals). These axons heavily innervate laminae IV and VI of area 18 in the kittens and adult cats. There are at least 2 differences however, between the terminal arborizations of the kitten and adult cat Y-axons: 1) The diameters of individual boutons on the kitten Y-axons are smaller than those on adult Y-axons (kitten mean = 1.3 μm, range = 0.5 μm - 2.3 μm; adult mean = 2.2 μm, range = 1.0 μm - 3.3 μm). 2) The distance between adjacent boutons (measured in 3-spatial dimensions) is less in the kittens than in the adults (kitten mean = 6.8 μm, range = 1.1 μm - 22.3 μm; adult mean = 11.9 μm, range = 0.9 μm - 24.9 μm). The postnatal development of the Y-cell projection to area 18 in the visual cortex therefore includes both growth of individual boutons and increased spacing between boutons. The latter result may be accomplished by bouton elimination, elongation of interbouton axon segments or a combination of these processes.

(Supported by NIH Grant EY03805, NATO Grant 0497/82 and the Alfred P. Sloan Foundation)

[1]Friedlander, M.J. Nature 300, 180-183, 1982.
[2]Tootle, J.S. and M.J. Friedlander Soc. Neurosci. Abstr. 10, 1984.
[3]Levay, S., M.P. Stryker and C.J. Shatz J. Comp. Neurol. 179, 223-244, 1978.

314.3 Enhanced phosphorylation of microtubule-associated protein, MAP_2, in the cat visual cortex during the critical period. C. Aoki and P. Siekevitz, Lab. Cell Biology, The Rockefeller University, New York, NY 10021

Kasamatu, et.al., Daniels, et.al. and Daw, et.al. reported that the local conc. of NE in the cortex can alter the degree of plasticity during the critical period (CP). We have been interested to know whether the cAMP cascade of events stimulated by NE could be involved, and have examined in the visual system the ontogenic changes in the enzymes which synthesize and degrade cAMP, the cAMP-dependent protein kinase, and the substrates for this kinase. Within each of five litters, kittens were either dark-reared (DR), light-reared (LR), or dark- and then light-reared (DL). DR kittens (n=7) were kept in complete darkness for 1-5 mos. with mothers, DL (n=8) were the littermates of DR but exposed to light for 3-17 hrs., and LR (n=7) were on 12D/12L cycle. Physiologically and behaviorally, DR has been shown to extend the CP of area 17 binocular cells (Cynader, et.al. and Mitchell, et.al), implying a separation of sensory-stimulated from general development. At 1, 2, 3, & 5 mos. area 17 and LGN were removed, and then homogenized in buffered isotonic sucrose. While some changes in the above enzymes were observed (Aoki & Siekevitz, 1983, Neurosci. Abs., p. 911), the most dramatic effect is the cAMP-dependent [^{32}P]-ATP phosphorylation of MAP_2. This protein was identified by mobility on SDS gels, by enhanced phosphorylation by cAMP, and by reaction on Western blots with monoclonal antibodies (provided by A. Matus and L.I. Binder). MAP_2, as well as Synapsin I, were phosphorylated in a cAMP-dependent manner under all three conditions of rearing. In all five litters, the visual cortex, but not the LGN, of DL kittens showed enhanced MAP_2 phosphorylation over DR (an increase of 25-350%, measured by cutting out MAP_2 bands and counting). The enhancement was independent of the addition of protein kinase, indicating that the enzyme was not limiting. In contrast, the phosphorylation of Synapsin I was not altered. Experiments are being done to see if MAP_2 concs. are changed by the rearing conditions. It has been reported that MAP_2 is found in dendrites and not in axons, that it is involved in the polymerization of tubulin and the association of microtubules with neurofilaments. Since phosphorylation of MAP_2 alters these activities, it may be that plasticity in the visual cortex involves a reorganization of dendritic spines and branching. Supported by NIH 1 RO3 EY04812-01 and NIH 5 T32 GM07524 Institutional Training Grant.

314.4 THE VISUAL CORTEX OF DARK REARED KITTENS: UNRESPONSIVE CELLS BECOME RESPONSIVE WITH INDUCED INCREASES IN EXCITABILITY. A.S. Ramoa*, M. Shadlen*, R.D. Freeman (SPON:P.A.Anderson), School of Optometry, Univ. of California, Berkeley, CA 94720

The visual cortex of dark-reared kittens is profoundly abnormal. Most cells are visually unresponsive or weakly responsive to visual stimulation and tend to be relatively unselective for basic stimulus properties such as orientation or direction. These changes could be due to complete functional or anatomical loss of various connections. On the other hand, inputs may be present but dysfunctional as a result of lack of visual stimulation. We have attempted to distinguish between these possibilities by artificially raising the level of excitability of cortical cells, which might reveal the existence of input that is otherwise subthreshold.

A three-barreled glass microelectrode with a sharpened tungsten wire projecting from one of the barrels was used for simultaneous single unit recording and ionophoresis of DL-homocysteic acid (DLH, 0.2M). DLH raises spontaneous activity of cortical neurons without affecting receptive field properties in normal cats. For the dark reared animals, a quantitative evaluation of responses during ionophoresis of DLH showed that:

1) All sampled cells could be driven by a moving bar of light, including those considered unresponsive prior to the administration of DLH.
2) For some cells, the only response was a suppression of the raised maintained discharge. This property generally cannot be revealed prior to DLH ejection due to the low spontaneous activity of most units.
3) Ionophoresis of DLH had no effect on selectivity to stimulus properties. Most cells were relatively unselective for the orientation and direction of moving slits.

These results show that most striate cortical cells in dark reared cats retain some kind of organized visual input. However, for many neurons this input allows responses which are just subthreshold or suppressive in nature. On the other hand, loss of stimulus specificity appears due to factors other than weakening of remaining connections. (EY01175)

314.5 MODIFICATION OF FUNCTION IN CAT VISUAL CORTICAL NEURONES INDUCED BY CONTROL OF THE CORRELATION BETWEEN POSTSYNAPTIC ACTIVITY AND VISUAL INPUT. Y.Frégnac, S.Thorpe, D.Shulz* and E.Bienenstock* (SPON: European Neuroscience Association). Laboratoire de Neurobiologie du Développement, Bat 440, Université de Paris XI, 91405 Orsay Cédex, France.

A likely mechanism of epigenesis in visual cortex is modification in synaptic transmission linked to correlation between pre- and post-synaptic activities (Hebb,1949;Frégnac and Imbert,1984). To test this hypothesis, a conditioning procedure was used: we artificially controlled the temporal correlation between presentation of a stimulus in the receptive field of the recorded cell and its postsynaptic activity. The aim of this study was to modify orientation preference and/or ocular dominance of single cortical neurones in anaesthetized (Althesin) and paralysed cats.

Ionophoretic techniques were used to clamp neuronal firing in association with different visual inputs: one stimulus of a given orientation or ocularity was presented while the cell's activity was maintained at a "low" level, and another one (of different orientation or ocularity) while the cell's firing was increased to a "high" level. Clamp of neuronal activity was achieved by varying the retention/ejection current of the potassium acetate or chloride extracellular recording electrode. Care was taken to avoid potassium accumulation and depression effects.

Control studies showed that preferred orientation is unaffected by the level of a constant current applied through the microelectrode. When the orientation conditioning procedure was applied (monocularly), significant changes in the visual response profile or in the relative preference for the two stimuli were found in more than 50% of the cases studied. These modifications were observed in normally reared cats as old as 5 months, but the clearest effects including significant changes of the preferred orientation were seen in deprived kittens recorded at 6 weeks of age.

Regarding ocular dominance, in spite of higher variability in control recordings, significant changes have been observed in the adult cat. Some cells retained the temporal pattern of discharge imposed during the conditioning. Similar experiments are carried out in kittens.

In general, the observed functional changes were consistent with Hebb's hypothesis: the stimulus presented when pre-post correlation was increased became more effective, while the stimulus seen during decreased correlation or block of activity became less effective.

This work has been supported by CNRS and DGRST grants.

314.6 PREMATURE EXPOSURE TO LIGHT DOES NOT ALTER THE COURSE OF SYNAPTOGENESIS IN THE PRIMATE STRIATE CORTEX. J.-P. Bourgeois* and P. Rakic. Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

Evidence from the literature indicates deficits of visual processing in premature human infants, while some experimental data in animals suggests an inductive effect of light on synaptic development. We tested the effects of precocious visual experience on the rate of synaptogenesis, ratio and size of various synaptic classes in the striate cortex of the rhesus monkey. Four fetuses were delivered by Caesarean section 3 weeks before term, which normally occurs at the 165th postconceptual day. The animals' eyes were opened and they were exposed to a 12-hour light/dark cycle in the primate nursery where they were raised. Four pairs of premature and control animals (delivered at term) were sacrificed at term, one, two, and three weeks after the expected day of birth, respectively. The striate cortex in the upper bank of the calcarine fissure was analyzed by electron microscopy. The density of synapses/100um^2 of neuropile, and the ratio and length of membrane densities of different classes of synapses were determined on photomontages spanning the entire cortical thickness.

We found that the synaptic density was similar in all layers in both experimental and control subjects, increasing from 15-20 contacts/100um^2 at term to 25-30 contacts/100um^2 of neuropile at three weeks. The percentage of synaptic contacts situated on somas (0-2%), dendritic shafts (20-30%) and spines (70-80%) were also similar in sublayers IVA, B, C in both groups of animals. A slight increase in the percentage of contacts on spines concomitant with a decrease in the contacts on shafts in sublayers IVA and B occurs between brith and 3 weeks in both controls and animals delivered prematurely. The length of synaptic contacts on dendritic spines and shafts in sublayers IVA, B, and C in normal and premature monkeys was not significantly different by the Mann-Whitney U-test. The finding that the rate of synaptogenesis, distribution and size of various classes of synaptic contacts in the visual cortex is unaltered by precocious visual experience suggests that the initiation and course of these cellular events may be determined by innate mechanisms. Whatever the effect of premature light exposure may be, it is not expressed in the synaptic paramaters measured in this study. Supported by EY02593 and NS19610.

314.7 SPATIO-TEMPORAL RELATIONS BETWEEN THE CELLS OF LAYERS 4 & 6 AND THEIR GENICULOCORTICAL AFFERENT INPUT DURING DEVELOPMENT. M.B. Luskin and C.J. Shatz. Dept. Neurobiology, Stanford Univ. Sch. Med., Stanford, CA 94305.

In the adult cat's primary visual cortex afferents from the lateral geniculate nucleus terminate mainly in layers 4 & 6. To find out more about how this pattern of connectivity is achieved, we have examined the relationship between the position of ingrowing afferents and cells destined for layers 4 & 6, at various times during the cat's 65 day gestation period. The afferent pathway was labeled by intraocular injections of ^{3}H-proline followed by transneuronal transport. Cells destined for layers 4 & 6 were identified by labeling with ^{3}H-thymidine via intrauterine injection on the days they are know to be generated: Embryonic day 37 (E37) to E43 for layer 4, and E30 to E35 for layer 6 (Neurosci. Abst., 8:3, 1982).

By E39, most layer 6 cells are postmigratory and positioned within the cortical plate (CP), while layer 4 cells have just begun to be generated. Afferents are confined to the optic radiations and cannot be detected either within the CP or underlying subplate (SP). Even 1 week later future layer 6 appears free of label, although afferents have invaded the SP. By E57, however, some afferents have grown into the lower half of the CP (future layers 5 & 6 from ^{3}H-thymidine labeling). The upper half of the CP consists almost entirely of future layer 4 at this age, yet no afferents can be detected there. By birth, the upper half of the CP is still immature, and is a heterogeneous mixture consisting of the postmigratory cells of layer 4 plus the cells of layers 2 & 3 that are still en route to their final positions external to layer 4. At this time, some of the afferents can be found not only in the lower but also in the upper half of the CP. It is not until the fourth postnatal week that the majority of cells comprising layers 2 & 3 reach their adult positon above layer 4; this time coincides with the onset of segregation of the geniculocortical afferents in layer 4.

These results indicate that in cat, as in monkey (Rakic, Phil. Trans. R. Soc., 278:245, 1977) geniculocortical afferents accumulate in the SP and wait at least 1 week before entering the CP. Thus it may be that the onset of the segregation of afferents into ocular dominance columns is signaled in part by the presence of a homogenous cortical layer 4 in which the majority of cells are postmigratory. (Supported by NIH grants EY02585 and NS07158).

314.8 THE DEVELOPMENT OF AXONAL ARBORS IN THE VISUAL CORTEX OF THE HAMSTER. J.R. Naegele and G.E. Schneider. Whitaker College and Dept. of Psychology, M.I.T., Cambridge, MA 02139.

The rapid postnatal formation of afferent axonal arbors in the visual cortex of the Syrian hamster was studied by inserting small pellets of HRP, adhering to the tip of a micropipette, into the optic radiations and processing the tissue using cobalt-intensified DAB histochemistry. Individual axons were traced with the aid of a drawing tube, and complete arbors were reconstructed from serial sections. Qualitative and quantitative comparisons were made of axonal branching at different ages.

On the day of birth (P0), HRP labelled afferent axons were located in the intermediate zone below the cortical plate. The axons were oriented parallel to the pial surface and frequently supported several short (<20 μm) filopodia, and one or more longer processes (branches) coursing toward the overlying cortical plate. Growth cones were located at the terminal ends of axons and on branches. On P3-P5, many more labelled axons were observed extending into the cortical plate where they formed small, rudimentary arbors oriented perpendicular to the pial surface. At this age, the arbors consisted of a central fiber with 4 or 5 primary branches often tipped with growth cones. By P12, larger and more complex terminal ramifications were observed within the cortex.

Comparisons with the morphology of presumptive geniculocortical axons in the mature animal indicate that the terminal arbors have attained an adult-like morphology by P24. Quantitative analysis of the distribution and lengths of branches demonstrates that afferent axons support multiple branches at various distances proximal to their end-arbor, at least until P5. On the average, during this period, each axon forms 1.5 branches per 100 μm of its length. However, widespread branching was seldom observed at P12. The disappearance of extraneous branches coincides with an augmentation of the terminal portion of the axonal arbor.

The age-related differences in the distribution and focalization of branches on afferent axons in the visual cortex may reflect an initial process of trial-and-error growth. Subsequent remodeling serves to improve spatial organization in the visual cortex; this process of maturation occurs before the eyes have opened on P14.
Support: NIH grants 5R01 EY00126 & 5P30 EY02621.

314.9 6-OHDA: PROTECTION AGAINST BEHAVIORAL EFFECTS OF MONOCULAR DEPRIVATION. B. Gordon, J. Moran*, P. Trombley*, and J. Soyke*. Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

Kasamatsu and his colleagues have reported that depleting norepinephrine (NE) in the visual cortex with 6-hydroxydopamine (6-OHDA) preserves the ability of a monocularly deprived (MD) eye to drive cells in the visual cortex (Science, 194:206, 1976; J. comp. Neurol., 185:139, 1979). If this protection from the effects of MD is behaviorally significant, 6-OHDA should also prevent the behavioral blindness in the deprived eye that would otherwise accompany MD. To test this prediction we compared the visual acuity of MD kittens treated with 6-OHDA intraventricularly with the acuity of monocularly deprived kittens that did not receive 6-OHDA. We trained the kittens to perform a visual acuity task at about 4 weeks of age. At about 5 weeks of age experimental kittens received 6-OHDA (4.8 mg/animal, over 6 days). Control kittens received vehicle solution intraventricularly or received no treatment at all. At about 6 weeks of age the right eye of all kittens was sutured shut for one week. When the deprived eye was opened at 7 weeks of age, 8 of 11 control kittens were blind when tested with the deprived eye. In contrast none of the kittens receiving 6-OHDA were blind when tested with the deprived eye. The difference between these groups was statistically significant ($p<0.05$, Fisher Exact Test). 6-OHDA had no effect on performance with the nondeprived eye.

We used high performance liquid chromatography to measure the amount of NE in the visual cortex of 5 experimental and 14 control animals. NE averaged 8 ± 2.8 ng/g (mean ± S.E.) in the experimental animals and 55 ± 4.9 ng/g (mean ± S.E.) in the control animals. Two of these controls were used in the behavioral experiments, twelve were used in other experiments.

We conclude that 6-OHDA protects vision through an eye that has suffered one week of deprivation. The fact that vision in the nondeprived eye was not impaired argues that 6-OHDA does not exert its effects by damaging connections from both eyes and thus decreasing binocular competition.

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314.10 NOREPINEPHRINE DEPLETION; RELATION TO VISUAL CORTICAL PLASTICITY. E. E. Allen, P. Trombley*, J. Soyke*, and B. Gordon. Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403

Kasamatsu, et al. (Science 194:206, 1976; J. Comp. Neurol. 185: 139, 1979; ibid., 163; J. Neurophys. 45:254, 1981), have obtained evidence that depletion of norepinephrine (NE) from the developing visual cortex of the cat results in a loss of neuronal plasticity. Adrien, et al.(C. R. Acad. Sc. Paris 295:745, 1982), Bear, et al. (Nature 302:245, 1983), and Daw, et al. (J. Neurosci., 1984, in press), however, have reported contrary results. According to Kasamatsu and his collegues, intraventricular administration of 6-hydroxydopamine (6-OHDA) to kittens eliminates the dominance of the nondeprived eye which would otherwise occur following a period of monocular deprivation (MD). In our hands 6-OHDA reduces, but does not eliminate, this shift in ocular dominance. Five control cats which received the ascorbic acid vehicle solution alone had a mean value of 7.8 (±2.2, S.E.) percent of visual cortical cells driven by the deprived eye, while 10 animals which received doses of 4.8 - 12.4 mg 6-OHDA had a mean value of 36.9 (±5.4, S.E.) percent. If, as Kasamatsu believes, this loss of plasticity is due to depletion of NE, then the dose-response relationship between 6-OHDA and the percentage of cortical cells driven by the deprived eye should parallel the relationship between 6-OHDA and cortical NE depletion. Kasamatsu and Pettigrew (1979; Fig. 5) present a series of dose-response relationships which indicate that the most pronounced effects of 6-OHDA on plasticity occur at doses greater than 10 mg, while doses of less than 5 mg have no effect; intermediate doses show limited protection from the effects of MD. Recent experiments in our laboratory have demonstrated, through the use of high performance liquid chromatography, that all doses of 6-OHDA from 0.1 - 4.8 mg result in approximately 90% depletion of cortical NE. Thus some effect of 6-OHDA other than depletion of cortical NE is probably responsible for its ability to protect against the effects of MD. In order to further clarify the relationships among 6-OHDA, NE, and neuronal plasticity, we are currently studying the physiological and behavioral effects of these low doses of 6-OHDA.

Supported by NIH grant EY04050-02.

314.11 HEMISPHERIC DIFFERENCES IN VISUAL EVOKED POTENTIALS ELICITED FROM CATS REARED WITH ALTERNATING MONOCULAR VISION. M. Shadlen* and R.D. Freeman (SPON: J.A. Anderson) Neurobiology Group, University of California, Berkeley, CA 94720.

Visual evoked potentials (VEPs) elicited by contrast reversal can be utilized to examine separate responses of the two hemispheres. Interhemispheric differences in activity can be enhanced by recording differentially between electrodes placed over each hemisphere. We have utilized this method of recording to compare hemispheric organization in normal cats and those reared with alternating monocular occlusion (AltMD).

We find that normal cats exhibit strong difference potentials (DPs) to contrast reversal restricted to one hemifield. The DP reverses polarity when stimuli are presented in the opposite hemifield, and is cancelled during full field stimulation. Waveforms are nearly identical for left and right eye stimulation, and exhibit summation when stimuli are presented binocularly.

Cats reared with AltMD show profound differences in DPs depending on the eye stimulated. The monocular response to the contralateral hemifield (uncrossed pathway) is extremely weak or absent. The binocular response is indistinguishable from the ipsilateral (dominant) monocular response. Full field stimulation to each eye produces strong DPs which are of opposite polarity for left and right eyes. Full field stimuli presented to both eyes produce cancellation.

Thus for cats deprived of normal binocular vision, a strong contralateral ocular dominance is reflected in one component of the VEP. Apparently, this organization is not evident from single unit recording. It has been reported that the developing kitten can utilize the temporal visual field (crossed pathway) prior to the nasal hemifield for optokinetic nystagmus and orienting behavior. Our result is consistent with these findings and suggests that binocular vision is necessary for development of a functional uncrossed pathway. The discrepancy between single unit recording and VEPs remains to be resolved.

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314.12 BINOCULAR DEPTH PERCEPTION IN CATS REARED WITH INTEROCULAR TORSIONAL DISPARITY. Paul G. Shinkman, Brian Timney, Michael R. Isley*, and Diane C. Rogers*. Univ. North Carolina, Chapel Hill, NC 27514, and Univ. Western Ontario, London, Canada N6A 5C2.

In the past few years we have reported the effects of rearing kittens with optically-induced torsional disparity between the two eyes' visual inputs, especially in terms of the interocular relationships of binocular cortical cells' receptive field properties. Kittens wore goggles fitted with small prisms that introduced opposite rotations of the images in the two eyes. The results of some of these experiments may be summarized as follows: after early experience with small interocular torsional disparities (16° total) the general physiological organization of visual cortex is relatively normal, except that the distribution of interocular disparities of binocular cells' preferred stimulus orientations is shifted away from zero and matches the experienced interocular disparity. With large interocular disparities (32° total) binocularity is severely disrupted, and the few remaining binocular cells do not show a compensatory change in preferred interocular orientation disparity on the average.

More recently we have studied the behavioral effects of prism goggle rearing. There are distinct parallels between the physiological and behavioral findings. We tested 32° goggle reared kittens using the jumping stand technique and found severe deficits in binocular depth perception, consistent with the loss of binocularity in visual cortex (Shinkman et al., Int. Cong. Physiol. Sci., 1983). The deficit is due neither to poor acuity, which is within normal limits, nor to artifacts of the rearing condition, since binocular depth discrimination of control kittens reared wearing 0° goggles is in the low normal range and is substantially superior to their monocular performance. We now report that 16° goggle reared kittens exhibit binocular depth perception similar to the control or 0° kittens. Binocular performance is superior to the discriminations made under monocular conditions. Thus the relatively normal cortical binocularity of kittens reared with small interocular torsional disparities is reflected in a correspondingly good perceptual capacity for binocular depth discrimination.

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NEURONAL DEATH: SYNAPSE ELIMINATION AND COMPETITION

315.1 MOTONEURON NUMBER INCREASES FOLLOWING AXOTOMY OF DEVELOPING SPINAL MOTONEURONS. P.B. Farel. Dept. of Physiology, Univ. N. Car. Sch. of Med. Chapel Hill, NC 27514.

While the phenomenon of naturally occuring death of motoneurons during development of the spinal cord has been well documented, the conditions which distinguish a motoneuron that will live from one that will die remain unclear. Most frequently, motoneuron survival has been thought to depend upon successful competition for a trophic substance that derives from the target organ. In the present work, however, the number of lumbar spinal motoneurons was found to increase following transient disconnection from the hindlimb.

Bullfrog (Rana catesbeiana) tadpoles between stages IV and XVIII were subjected to unilateral transection of the three ventral roots that provide innervation to the hindlimb. Six weeks postoperatively, the lumbar enlargement was removed and prepared for methacrylate (before st. VII) or paraffin embedment and sectioned at 2 um and 8 um respectively. Motoneurons on the two sides of the spinal cord were counted in alternate paraffin sections and in every eighth methacrylate section. Immature motoneurons in the LMC were identified by the presence of large, pale nuclei containing clumps of basophilic material. Mature motoneurons were identified by their characteristic shape and the presence of a single large nucleolus. That both these profiles were motoneurons was confirmed in several experiments by noting their filling following application of HRP to the ventral root. Neither nuclear nor nucleolar size differed on the operated and unoperated sides.

The number of motoneurons on the operated side exceeded that on the control side by as much as 100% before st. IX. In tadpoles operated between stages XI and XIV, a 25% increase in motoneurons on the operated side was found.

Preliminary evidence indicates that these increases persist beyond the end of the period in which naturally occurring motoneuron death is found. The possibility is thus raised that the target organ conveys to the motoneuron a signal that results in death of the motoneuron. Disconnection from the target during the period when the signal would normally be received appears to result in sparing of the motoneuron.

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315.2 REGIONAL VARIATION IN NATURALLY OCCURRING GANGLION CELL DEATH PARALLELED BY MICROGLIAL INVASION IN THE POSTNATAL CAT RETINA. H.E. Pearson[1], B.R. Payne[2] and T.J. Cunningham[2]. Depts. of Anatomy, Temple Univ. Med. Sch.[1] and Med. Coll. of Pa.[2], Philadelphia, Pennsylvania.

The generation and maturation of ganglion cells in cat retina show regional variation such that development of cells in central retina precedes that of cells in the periphery. We have already demonstrated that naturally occurring neuron death contributes to postnatal development of the ganglion cell layer in peripheral retina of the cat (Pearson et al, 1983). We have now expanded this study to investigate the extent of naturally occurring ganglion cell death in central retina. Our aim was to determine whether any regional variation is present in the timing of this process and to investigate the relationship of ganglion cell death to the invasion of phagocytic microglia cells.

Retinae were obtained from aldehyde-perfused kittens between the ages of 2 and 10 days postnatal. From each retina, samples from both central and dorsal peripheral regions were embedded in plastic and sectioned at one micron. From each sample, regularly spaced sections were stained with toluidine blue and examined for the presence of normal ganglion cells, degenerating cells and microglia. The surface areal density of each type of profile was determined.

Counts of degenerating cells show a gradual loss of ganglion cells over the ages examined. In constrast, counts of normal ganglion cells show a sharp decline in a more restricted period - between 2 and 3 days postnatal in central retina and between 8 and 10 days in peripheral retina. The results suggest that cell death in central retina precedes that in far peripheral retina by about a week. Microglia were present at all ages examined, but in both central and peripheral retina, were most abundant at the time of greatest ganglion cell loss. From these results we conclude firstly, that there is regional variation in naturally occurring neuron death in the postnatal cat retina. Secondly, increased degeneration of neurons in the ganglion cell layer is accompanied by an increase in the density of phagocytic microglia, so that the density of degenerating profiles is maintained at a near constant level. We are currently investigating whether increased neuronal death serves as a trigger for microglial mobilization in cat retina.

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315.3 QUANTITATIVE CYTOLOGY OF THE POSTNATAL DEVELOPMENT OF NIGROSTRIATAL DOPAMINE NEURONS IN THE RAT: RELATIONSHIP TO CELL DEATH AND FUNCTIONAL CONNECTIVITY. M. Saji*, T.H. Joh, and D.J. Reis (SPON. R. Ross), Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

We sought to determine using quantitative cytochemical methods whether dopaminergic neurons of the substantia nigra pars compacta (SNc) of rat,undergo postnatal cell death in relationship to: (a) the development of functional connections; and (b) postnatal accumulation of immunoreactive tyrosine hydroxylase (TH) in the nigrostriatal pathway.

In adult rats, the SNc on each side contains 10,242 ± 670 neurons. Of these, 8558 ± 433 or 84% contain TH revealed by using saturating staining conditions. After injections of HRP filling 81% of the caudate nucleus, 5930 ± 388 SNc neurons were labeled (representing 58% of total and 70% of TH-labeled neurons). By double-staining 95% of HRP-labeled neurons contained TH. The range, mean size, and distribution of SNc neurons with or without TH or transported HRP were identical. Lesions of ascending bundle resulted in retrograde death of approximately 65% of SNc neurons within 12 d. During postnatal (P) development from P1 to adult, the length of SNc increased 1.8x. The total number of neurons at birth (9442 ± 330) did not differ significantly from those of adults. The number of neurons at P1 with TH (at saturation) was 5960 ± 590 or 66% of total. The number of TH-neurons gradually increased reaching adult values by P5. With sub-saturating staining, the major changes in TH content occurs between P5 and P11. The number of HRP labeled neurons was 1910 ± 56, or 20% of total, at P1, increasing slowly P1-P2 and rapidly between P2-P5 reaching near adult values at that time. The results indicate: (a) over 90% of neurons in SN contain TH, but only 70% of these project to ipsilateral striatum; (b) that 80% of the capacity of nigrostriatal neurons to transport HRP develops postnatally between P2-P5 and in advance of full biochemical maturation. We conclude: (c) Competition for target organ is not associated with programmed postnatal cell death in the dopaminergic nigrostriatal system; (d) functional contact of SNc neurons with target elicits the final accumulation of TH. These results suggest that the completion of neural connectivity is required for the full phenotypic expression of TH in the nigrostriatal system. (Supported by Grant HL18974).

315.4 THE EFFECT OF TETROTOTOXIN ON THE DEATH OF MAMMALIAN RETINAL GANGLION CELLS. Stuart A. Lipton and Paul Harcourt*. Dept. of Neurology, Harvard Medical School and Div. of Neuroscience, Children's Hospital Medical Center, Boston, MA 02115.

To look for factors that might be important in natural cell death, we studied ganglion cells in cultures of dissociated retina. This approach afforded control of the extracellular environment and facilitated electrical recordings. Our preliminary results indicate that maintained regenerative activity may be important in preventing cell death.

After labelling the ganglion cells by retrograde transport of fluorescent dye, retinas of 7 day old rats were dissociated and cultured as previously described (Leifer, Lipton, Barnstable & Masland, Science 1984;224:303-6). Ganglion cells were identified by the presence of retrograde label and/or with a monoclonal antibody to Thy-1. In culture, identified ganglion cells were found as solitary cells or within small clusters of other retinal cells. Ganglion cells in clusters remained viable for up to 2 1/2 weeks and solitary cells one week (Leifer et al., ibid). Recordings with intracellular electrodes revealed that both solitary and clustered ganglion cells fired action potentials upon depolarization. These action potentials were blocked by 0.1-1 μM tetrototoxin (TTX). To avoid injury current on penetration with microelectrodes, recordings were also made with extracellular patch pipettes. Using this technique, solitary ganglion cells only rarely displayed spontaneous spikes, whereas about half of the recordings (N=46) from ganglion cells found in clusters had spontaneous action potentials. In cultures incubated in 1 μM TTX for the first 24 hrs after plating, there was a 50% decrease in the number of ganglion cells found in clusters compared with control cultures containing normal media without TTX. On the other hand, TTX had no effect on the number of solitary ganglion cells surviving in culture or on the percentage of solitary ganglion cells capable of regenerating processes on plain glass or on glass coated with polylysine, collagen, or antibody to Thy-1 (Leifer et al., ibid). Thus, when spontaneous activity was prevented, a significant proportion of the ganglion cells in clusters died, but there was no increased death of solitary cells. It would thus appear that the maintenance of spontaneous regenerative activity in a population of ganglion cells may be important in preventing their death. In vivo, natural death occurs in a large number of retinal ganglion cells that are the same age as these cultured cells. Whether our results are a reflection on the normal process of cell death remains to be settled.

315.5 REDUCTION OF NATURALLY-OCCURRING MOTONEURON DEATH IN THE SPINAL CORD OF THE MOUSE MUTANT, MUSCULAR DYSGENESIS. R. W. Oppenheim, J. A. Powell* and L. J. Standish , Dept. of Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27103 and Depts. of Biology and Psychology, Smith College, Northampton, MA 01063.

Muscular dysgenesis (mdg) is a lethal autosomal recessive mutant characterized by the absence of all neuromuscular activity during embryogenesis; respiratory activity is absent and the mice invariably die at parturition. The affected embryos exhibit excessive intramuscular branching of nerves, increased numbers of acetylcholine receptors (AChR) and AChR clusters per myofiber, increased foci of acetylcholinesterase staining of myofibers and increased levels of choline acetyltransferase activity. Many of these changes have also been reported in chick embryos chronically treated with neuromuscular blocking agents that suppress motility. The suppression of motility in the chick also results in a near-total reduction of naturally-occurring motoneuron (MN) death. Thus, in this context, the mdg mutant is of particular interest because the defect appears to effect excitation-contraction coupling but not synaptic transmission.

Motoneuron survival was studied in the lumbar and thoracic spinal cords of mutant (mdg/mdg) and control (+/mdg?) embryos from embryonic day (E) 13 to E18. Cell counts of both healthy and degenerating MNs and dorsal root ganglion (DRG) cells were made through all lumbar and thoracic segments. Cell size, nucleolar measures and spinal cord volumes were also determined.

There was a 59% decrease of lumbar and a 53% decrease of thoracic MNs in the control (+/mdg?) embryos between E13 and E18 indicating a significant normal MN death. At all ages the mdg/mdg embryos had significantly more healthy and significantly fewer degenerating MNs than controls. By E18 this difference was approximately 45% (healthy) for both thoracic and lumbar MNs. There were no differences in the number of DRG cells or in any of the other morphometric parameters examined between mdg/mdg and +/mdg? embryos. Thus, the absence of muscle contractions (but not synaptic transmission) in the mdg mouse mutant is associated with a large reduction of naturally-occurring MN death.

315.6 THE NUMBER, SIZE, MYELINATION, AND REGIONAL VARIATION OF AXONS IN THE CORPUS CALLOSUM AND ANTERIOR COMMISSURE OF THE DEVELOPING RHESUS MONKEY. A-S. LaMantia and P. Rakic. Sec. Neuroanatomy, Yale Univ. Sch. Med. New Haven, CT 06510.

Axons in the corpus callosum (CC) and anterior commissure (AC) were studied in 12 pre- and postnatal rhesus monkeys. The CC or AC was dissected as a single 1 mm thick mid-sagittal slice, embedded in plastic and after polymerization, divided into smaller blocks. The surface area of each block was determined from 1 um sections and selected regions were sectioned for electron microscopy. The density, diameter, distribution, and total number of axons was determined for the entire CC and AC. Mature monkeys have about 45×10^6 axons in the CC and 3.5×10^6 in the AC, 94% of which are myelinated. Small and medium sized myelinated axons (0.5-4 um) are present throughout the CC, while large myelinated fibers (4-10 um) are limited to the body and splenium; unmyelinated fibers are concentrated in the genu. Axons of the CC are produced in excess prenatally and eliminated postnatally. Two months before birth the number of CC axons is 100×10^6, twice the adult number. At term the number of axons reaches a maximum of 150×10^6, 3X the adult value. Regional differences in axon size and myelination emerge around birth. After birth, axons are eliminated at a rate of 425,000/day, attaining adult levels in the 6th month when an average of 25% are still unmyelinated. Since the number of myelinated axons never exceeds the adult level and degenerating myelinated axons are not observed during the period of loss, most axons must be eliminated before they reach their final size and become myelinated. Axonal loss is not related to the formation of callosal columns which are formed well before elimination begins (Goldman-Rakic, '82, NRP Bull. 20:520). In contrast, axon loss coincides with a rapid increase in synaptic density in the neocortex; a gradual decrease in synaptic density only occurs after the adult number of axons has been reached (Bourgeois, Zecevic & Rakic, unpub.). The AC also contains an exuberant number of axons during development. However, the adult level is reached by the 2nd postnatal month, 4 months earlier than in the CC. This difference may reflect an earlier onset and faster tempo of maturation in cortical areas related to the AC. Both collateral retraction and cell death have been suggested as mechanisms which underlie axon elimination in the developing mammalian CNS. The relative contributions of these mechanisms to the development of the primate cerebral commissures remain to be determined. Supported by the NIH.

315.7 TRUE BLUE REVEALS THE MAINTENANCE OF NUMEROUS AFFERENTS TO THE RAT ADRENAL MEDULLA. L.L. Ross, A.J. Smolen and L. McCarthy.* Dept. Anatomy, The Med. Coll. PA, Phila. PA 19129.

Previously we have used HRP to retrogradely label preganglionic neurons which project to one adrenal medulla. Prior to postnatal day 20, many neurons in the spinal cord are labeled both ipsilateral and contralateral to the injected adrenal medulla. By day 25, HRP labels far fewer cells ipsilaterally and virtually none contralaterally. From these observations we concluded that, in common with other areas of the developing nervous system, there is an early exuberant projection of preganglionic axons to the adrenal medulla followed by a loss of axons and a progressive restriction of the afferent input.

It is generally thought that the loss of exuberant projection is due to cell death of inappropriately projecting neurons or to retraction of their axons. To examine this, we repeated the injection protocol using True Blue, a longer-lasting, more efficient retrograde labeling agent than HRP. We injected True Blue on day 10. By day 15 the pattern of True Blue labeling was essentially the same as that demonstrated by HRP, although the total number of cells labeled by True Blue was much greater, We observed many labeled cells both ipsilateral and contralateral to the side of the injected adrenal. By day 25, there was no loss of True Blue labeled cells when compared to the number labeled at day 15. These observations are consistent with the hypothesis that the inappropriately projecting axons appear to be withdrawn and the neurons are sustained by other targets to which they project.

As a further verification of this hypothesis, we injected True Blue into one adrenal gland on day 25 and counted the number of labeled neurons on day 29. We again observed many labeled neurons on both sides of the spinal cord. Thus, preganglionic neurons do not completely withdraw their axons from the adrenal medulla during postnatal development. These observations demonstrate that the early exuberant projections to the adrenal medulla are maintained beyond the immediate postnatal period when preganglionic inputs were presumed to have been lost.

We propose that the loss of exuberance in the nervous system may not be qualitative, whereby all of the inappropriate afferents are lost, but rather it may be quantitative, whereby the inappropriate afferents are maintained but reduced in relative importance by either partial retraction or by unequal overgrowth of the more appropriate afferents.

315.8 SYNAPTIC DISTRIBUTION ON SUPERIOR CERVICAL GANGLION CELL SOMATA AND DENDRITES IN DIFFERENT MAMMALIAN SPECIES. D. Purves, C.J. Forehand and J.W. Lichtman, Dept. of Physiol. and Biophys., Wash. Univ. Sch. Med., St. Louis, MO 63110.

In some mammalian autonomic ganglia neurons receive very little preganglionic innervation on their cell bodies, whereas in other ganglia principal neurons receive dense somatic innervation. Since neurons with few cell body contacts generally have complex dendritic arbors, and vice-versa, it occurred to us that the distribution of synapses on cell bodies of homologous neurons might vary systematically as a function of neuronal geometry. We therefore compared the innervation of cell bodies and dendrites in mouse, hamster, rat, guinea pig and rabbit superior cervical ganglia (SCG) in which the principal neurons differ both in dendritic complexity and in the degree of preganglionic convergence onto ganglion cells (Purves and Lichtman, Soc. Neurosci. Abstr 9: 320, 1983).

The somatic innervation of these neurons does indeed vary across species hand in hand with dendritic complexity. Thus mouse SCG cells, which have 4-5 dendrites and are innervated by 4-5 preganglionic inputs receive about 9.4 synaptic contacts per 1000 µm of cell body membrane profile. On the other hand, rabbit SCG cells, which on average bear a dozen primary dendrites and are innervated by about 15 preganglionic axons receive only about 1.8 synaptic contacts per 1000 µm of cell body membrane. An intermediate number of somatic contacts was observed in the other species, consistent with their intermediate dendritic complexity. Synaptic density in the ganglia, whether expressed per unit area or per cell profile, did not differ significantly among the various species. Therefore, the relative number of synapses made on cell bodies decreases as dendritic complexity and input convergence increase.

Although the reason for these differences in synaptic distribution is not clear, the systematic variation of synaptic distribution on target neurons as a function of dendritic complexity suggests a general rule of innervation which bears further investigation.

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315.9 EFFECT OF DENERVATION ON DENDRITIC GROWTH IN THE NEONATAL SUPERIOR CERVICAL GANGLION. J. Voyvodic* and D. Purves, (SPON: J. Lichtman). Dept. of Physiol. and Biophys., Washington Univ. Sch. of Med., St. Louis, MO 63110.

The role of preganglionic innervation in the postnatal development of dendrites was investigated in the superior cervical ganglion (SCG) of the hamster. Ganglion cell morphology was analyzed quantitatively using a computer assisted measuring procedure after intracellular injection of horseradish peroxidase (HRP).

Hamster SCG cells are morphologically immature at birth and undergo a marked postnatal increase in the length of their dendrites. Thus neonatal SCG cells (from animals 0 to 3 days old) had an average of 6.0 primary dendritic processes, and an average total dendritic length of 200 µm; the mean radial distance from the cell soma to the furthest dendritic process was 39 µm (N=23 cells). The SCG cells of adult hamsters had an average of 7.4 primary dendrites with a total dendritic length of 1224 µm and an average maximum extension of 182 µm (N=54 cells). Thus the total dendritic length increases about 6 fold after birth and the overall size of the dendritic arbor increases 4-5 times.

The influence of preganglionic axons on the postnatal development of dendritic geometry was assessed by cutting the cervical sympathetic trunk (CST) within one day of birth; the animals were then examined at 4 weeks of age. Reinnervation was prevented by ligation and resection of the proximal end of the CST. Complete denervation was verified by intracellular recording at the time of sacrifice; none of the cells showed a synaptic response to stimulation of the distal stump of the preganglionic trunk. In contrast, all control cells showed suprathreshold responses to preganglionic stimulation.

At 4 weeks of age the ganglion cells denervated since birth had an average total dendritic length of 899±347 µm (SD, N=21 cells in 8 ganglia); this is an increase of 4.5 fold since the time of denervation. Unoperated control cells at this age had an average total dendritic length of 1097±453 µm (SD, N=26 cells in 9 ganglia). Similarly there was only a slight difference between denervated and control cells in the number of dendrites or the maximum extension of their dendritic arbors. Therefore preganglionic fibers appear to play a relatively minor role in the postnatal development of dendrites in this system.

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315.10 INNERVATION OF RABBIT CILIARY NEURONS: ELECTRON MICROSCOPIC ANALYSIS OF THE DISTRIBUTION OF SYNAPTIC BOUTONS FROM HORSERADISH PEROXIDASE-LABELED PREGANGLIONIC AXONS. C.J. Forehand and D. Dill*, Dept. of Physiol. and Biophys., Wash. Univ. Sch. of Med., St. Louis, MO 63110.

The number of inputs a neuron receives in the rabbit ciliary ganglion is directly correlated with the number of target cell dendrites (Purves and Hume, J. Neurosci. 1: 441, 1981). Moreover, the innervation of geometrically complex (multiply innervated) neurons by individual preganglionic axons is regional: the synaptic contacts made by an axon onto such neurons are limited to a portion of the postsynaptic surface that usually includes the cell body and some, but not all, of the dendrites (Forehand and Purves, J. Neurosci. 4: 1, 1984). To explore the degree to which dendritic regions are exclusively innervated by a given axon, we have examined the ultrastructural distribution of boutons from individual preganglionic axons that have been intracellularly marked with horseradish peroxidase (HRP).

Camera lucida drawings of the HRP-labeled axons (visualized with diaminobenzidine) were made from 40 µm vibratome sections; serial thin sections were then obtained for electron microscopic examination. Eight neurons that were innervated by a labeled axon and possessed at least two dendrites were partially reconstructed to examine the deployment of labeled synaptic boutons on their surface. Each of these 8 cells had a minimum of two inputs since both labeled and unlabled synapses were observed.

At least one proximal dendrite extending from each of the 8 cells was contacted by only labeled or only unlabeled boutons over the distance reconstructed (10-60 µm). In 5 cases the dendritic segment received only labeled boutons, and thus was exclusively innervated by a single axon. On the other hand, six of the cells received both labeled and unlabeled boutons on one of the dendritic segments examined.

These observations indicate that the regional innervation of rabbit ciliary neurons by individual axons can, in some cases, reflect exclusive innervation of a dendritic segment by a single axon; however, the coexistence of at least two inputs on some dendrites indicates that an axon's terminals do not always gain exclusive dominion over the region of the postsynaptic surface they innervate.

(Supported by NIH grant NS18629 to Dr. Dale Purves. C.J.F. is a postdoctoral fellow of the MDA.)

315.11 THE GROWTH OF CATECHOLAMINE- AND ACHE-CONTAINING FIBERS INTO NEOCORTICAL TRANSPLANTS. J. Park*, R.J. Clinton*, F.F. Ebner, Div. of Biol. and Med., Brown University, Providence, R.I. 02912

When embryonic neocortex is implanted into the neocortex of adult BALB/c mice, many neurons and glia survive, presumably for the lifetime of the host animal. AChE-positive fibers from the host brain start to grow into transplants within one week and they achieve a density equivalent to the host cortex by two months after transplantation. Only a few thalamic fibers grow into the transplants from the host brain at any time after implantation. One hypothesis to account for the poor ingrowth of thalamic fibers into the transplants is that the early arrival of cholinergic and catecholaminergic (CA) fibers sets up conditions that actively inhibit thalamic fiber elongation into the donor cell matrix. We studied the onset of CA fiber growth from the host into the transplants, using the glyoxylic acid histofluorescence technique as modified by de la Torre. CA fibers were not demonstrable within the transplants from E17-19 donors for 30 days, even after pargyline pretreatment. Transplants from younger donors (E12-14) showed detectable fluorescence slightly earlier, about 21 days after transplantation. Longer survival periods led to modest ingrowth of CA fibers by 6-8 weeks, but their density never approached that of host cortex even after 6 month survivals. There is, however, a definite buildup of histofluorescent fibers on the host brain side of the interface region. No detectable change in the ingrowth of CA-containing fibers was produced by soaking the donor tissue in 10^{-4}M NGF for 10 min. prior to implantation. No change in the ingrowth of AChE+ fibers was seen after implanting into hosts depleted of CA by DSP4 treatment.

We conclude that the host CA fibers are relatively slow to grow into neocortical transplants under our conditions, and hence, that CA fiber ingrowth per se is not directly responsible for preventing thalamic fiber invasion of the transplanted tissue. We do not know whether the release of CA's from fibers close to, but outside of the transplant can diffuse across the border to influence the development and mature characteristics of neurons and glia in the transplants. Cholinergic fibers, therefore, emerge as the only known fiber system to grow into the transplants at an early stage and to achieve densities throughout the transplants that are equivalent to normal adult or host cortex (Supported by NIH grant #NS13031).

315.12 SPROUTING IN GRANULOPRIVAL CEREBELLAR CULTURES IS NOT PREVENTED BY SUPPLEMENTATION WITH GLUTAMATE OR GABA. F.J. Seil and A.L. Leiman*. Neurology Research, VA Med. Ctr. and Dept. of Neurology, Oregon Health Sciences Univ., Portland, OR 97201, and Dept. of Psychology, Univ. of Calif., Berkeley, CA 94720.

Cerebellar explants derived from neonatal mice exposed to cytosine arabinoside (Ara C) for the first 5 days in vitro (DIV) to destroy granule cells undergo a remarkable sprouting of Purkinje cell recurrent axon collaterals (Seil, F.J. et al., Brain Res. 186:393, 1980). In order to determine if supplementation with the granule cell putative neurotransmitter, glutamic acid, might prevent such sprouting, or if presence of large amounts of the Purkinje cell neurotransmitter, GABA, might suppress recurrent axon collateral sprouting, cerebellar cultures were explanted with 5 µg Ara C/ml medium plus large concentrations of the amino acids for the first 5 DIV. After 5 DIV, the cultures were continuously exposed to 10^{-3}M or $2x10^{-3}$M concentrations of L-glutamate, D-glutamate or GABA incorporated into the nutrient medium.

The excitatory effects of the glutamic acid isomers after bath application to cerebellar cultures have been reported (Seil, F.J. et al., Brain Res. 159:431, 1978; 161:253, 1979). GABA produced a reversible inhibition of cortical spontaneous electrical discharges. Cultures exposed to Ara C plus the amino acids were not appreciably different morphologically from cultures treated with Ara C alone when viewed by light microscopy in whole mount preparations fixed after 15 DIV and stained with silver. Neither the glutamic acid isomers nor excess GABA prevented Purkinje cell axon collateral sprouting. It is concluded that loss of putative neurotransmitter consequent to granule cell destruction is not the trigger for Purkinje cell axon collateral sprouting in the granuloprival cerebellar culture model, but that such sprouting is more likely due to an alteration of some trophic interaction between Purkinje and granule cells.

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REGENERATION IV

316.1 GANGLION CELL AXONS REGENERATE ALONG SEGMENTS OF PERIPHERAL NERVE TRANSPLANTED INTO THE RETINA OF ADULT RATS. K.-F. So and A.J. Aguayo. Neurosciences Unit, Montreal General Hospital, and Department of Neurology, McGill University, Montreal, Quebec, Canada H3G 1A4

Because nerve cells in several different regions of the mammalian brain and spinal cord have been shown to regrow axons along peripheral nerve transplants we have now investigated the response of retinal ganglion cells to the focal implantation of a segment of peripheral nerve into the eye.

In adult Sprague Dawley rats a 2 to 4 cm long segment of autologous peroneal nerve was removed from the thigh and used for grafting. One end of this peripheral nerve graft was transplanted into the retina through a small scleral incision made in the superior temporal quadrant of the eye, approximately 4 mm behind the limbus, and secured with 10-0 sutures. The rest of the nerve graft was placed over the roof of the orbit and skull, its other end tied to the subcutaneous tissues overlaying the occipital bone. Animals were sacrificed 1 to 3 months after grafting. Two days before sacrifice the caudal tip of the graft was dissected to permit the application of horseradish peroxidase (Sigma VI) at a concentration of 30-50%. The entire retina was removed, processed and examined as a whole mount to determine the presence and distribution of retrogradely labelled neurons.

We have found horseradish peroxidase labelled ganglion neurons of different cell diameters in an area of the retina that is peripheral to the site of grafting. Such distribution suggests that the regenerating axons originate from fibers damaged along their retinal course to the optic disc. These findings indicate that axons of retinal ganglion neurons are capable of extensive regrowth after damage. Non-neuronal components of the peripheral nerve graft appear to influence the expression of the regenerative capability of these central nervous system neurons. (Supported by Medical Research Council of Canada and The Croucher Foundation of Hong Kong. K.-F. So is on leave from Dept. of Anatomy, Univ. of Hong Kong).

316.2 REGENERATION OF LONG SPINAL AXONS INTO PERIPHERAL NERVOUS SYSTEM GRAFTS IN THE CAT. D.J. Sceats*, W.A. Friedman, G.W. Sypert, and W.E. Ballinger*. VA Medical Center and Depts. of Neurological Surgery, Neuroscience, and Pathology, Univ. of Florida, Gainesville, FL 32610

In cats, sciatic nerve autografts were made to a left rostral (T2-4) and caudal (L2-4) hemisected spinal cord and studied using histological techniques. Aspiration lesions were made in the left side of the cord and the sciatic divided and sewn to the dura at both sites with the distal end tunneled subcutaneously. Cats were studied with standard histological techniques at 73, 146, and 149 days postoperatively. Common to all was axonal growth the entire length of the grafts. The amount of axonal regeneration appeared to be related to the amount of interface. The interface showed proliferation of fibroblasts, astrocytes, arachnoid cells, Schwann cells, microcyst formation and much intermingled collagen. The perineurium appeared thickened and was contiguous with the dura mater. At 73 days signs of late Wallerian degeneration were combined with new axonal growth. At 146 and 149 days the acute response had resolved, but some areas had undergone endoneurial fibrosis. The graft tip appeared similar to a neuroma.

Thus far, four cats have undergone investigation with horseradish peroxidase (HRP). In the first two, a concentrated solution of HRP was applied to the cut ends of the grafts and the graft isolated on a rubber dam for one hour. Two days later, the animals were sacrificed by perfusion with gluteraldehyde phosphate buffered sucrose. Five pieces of tissue were serially sectioned including: sensorimotor cortex, the entire midbrain and brainstem complex, a 3.5 cm segment containing the rostral graft, a 3.5 cm segment containing the caudal graft and the 3.5 cm immediately distal to the caudal graft. Alternate sections of the tissue were processed with a TMB technique. In contrast to previously reported grafts at C-8 in the rat, (Richardson et al., J Neurocytol 13:165-182, 1984) no labeled neurons were found in the cortex or brainstem, even though the grafts were demonstrated to have good connectivity and many local neurons were labeled. Neurons were labeled at least six cm caudal to the caudal graft. In two subsequent cats the cortex and brainstem were not studied, with attention directed to the graft interface and the spinal cord. HRP was applied only to the caudal grafts, and it was noted that the degree and quality of interface was related to the numbers of neurons labeled. Neurons up to seven cm distal to the graft site were labeled within the gray matter of the cord.

316.3 GROWTH OF CNS AXONS THROUGH PERIPHERAL NERVE IMPLANTS IN THE CAT. R. P. Dum and C. G. Salame*, Dept. of Neurosurgery, SUNY at Upstate Medical Center, Syracuse, NY, 13210.

Rat central nervous system (CNS) axons have shown a remarkable capacity to grow through implanted pieces of peripheral nerve (David & Aguayo, Science 214:931, 1981). This study examined the capacity of CNS neurons in the cat to sprout and elongate through peripheral nerve grafts.

In young adult cats, a 64-80mm autograft of the superficial peroneal nerve was implanted into the brainstem, led extraspinally and inserted into the spinal cord (C5-C6). Otherwise the neuraxis was left intact.

Four to nine months later, cats were anesthetized with barbiturates and bipolar cuff electrodes were placed on the nerve graft. Short trains of electrical stimuli evoked observable contractions in various face and shoulder muscles. Likewise, the diaphragmic EMG was inhibited by the stimulation of the nerve graft. Control stimulation applied directly to the tissue surrounding the cuff electrodes did not reproduce the effects of nerve graft stimulation.

HRP applied to the cut ends of the transected nerve graft was used to retrogradely label neurons whose axons entered the graft. After TMB processing, labeled neurons were found in both the brainstem and spinal cord (mean 280, range 185-374). The length of the nerve graft traversed by these newly growing axons was between 15 and 48mm. In the brainstem, all labeled neurons were within 2.0mm of the implant. The following nuclei were labeled: cuneate, dorsal motor nucleus of the vagus, gracilis, lateral tegmental field, medial solitarius, and retroambiguus. In the spinal cord, labeled neurons were found as far as 21.0mm from the implant site. Labeled neurons were found within laminae 1, 4-10. Dorsal root ganglion (DRG) cells were labeled primarily in ipsilateral DRGs adjacent to the implant site.

A number of conclusions can be drawn from this study. First, a variety of neuronal types within the cat CNS are able to sprout and elongate their axons for up to 5cm through peripheral nerve grafts. Thus, the failure of CNS regeneration is not due to an intrinsic inability of CNS neurons to regrow. Second, these axons can conduct action potentials which produce transsynaptic effects either through axon collaterals activated by antidromic stimulation or by the formation of new synaptic connections. Third, the ability of CNS neurons to regrow depends on their proximity to the implant site.

316.4 CHARACTERISTICS OF CENTRAL NERVOUS SYSTEM (CNS) AXONAL REGENERATION INTO SCIATIC NERVE IMPLANTS IN RATS. C.G.Salame* and R.P.Dum, (SPON:G.H.Collins). Dept. of Neurosurgery, SUNY at Upstate Medical Center, Syracuse, NY13210.

Peripheral nervous system grafts are known to promote elongation of CNS axons. Extensive CNS axonal elongation into sciatic nerve grafts has been histologically documented in rats (David and Aguayo, Science 214:931; 1981). This study examines the physiological characteristics of CNS axons growing through sciatic nerve grafts and the regenerative capabilities of different CNS neurons, in adult rats.

In thirteen female rats (200-225gms), one end of an autologous sciatic nerve was implanted in the medulla. The distal end was led extraspinally and implanted in the ipsilateral cervical cord (C4).

Four to eight months after implantation, electrical stimulation of the graft evoked EMG activity in a variety of head and neck muscles in 6/8 rats. In rats where diaphragmatic EMG was recorded, electrical stimulation of the graft during inspiration resulted in a change of the ongoing EMG in 4/7 rats. This change in EMG activity consisted mainly of potentiation of the EMG response in the ipsilateral diaphragm (3/4). No spontaneous neural activity was recorded from the grafts even with extensive manipulation of the skin.

After the recordings were done, the grafts were cut and their ends soaked in horseradish peroxidase (HRP) dissolved in 2% DMSO. When the graft was firmly implanted in the neuraxis, the average count of HRP labeled neurons in the brainstem and spinal cord was 563 (183-1503) and 398 (17-1335) respectively. Most labeled neurons were concentrated within ±4mm of the implant site, but some were observed up to 9mm from either end of the graft. In the brainstem, labeled nuclei included the dorsal motor nucleus of the vagus, ambiguus, hypoglossal, lateral reticularis, gigantocellularis, raphe and subcoeruleus. The contralateral red nucleus and lateral pontine reticular nucleus were labeled as well. In the spinal cord, labeled neurons were found in laminae 4-8 and lamina 10. They tended to be ipsilateral to the graft near the site of implant, but were found contralaterally, farther away.

These results suggest that regenerating CNS axons within sciatic nerve grafts in rats are able to conduct action potentials and possibly establish functional synapses on CNS neurons, and show that 1) regeneration is more prominent in neurons close to the site of the peripheral implant, and 2) many different kinds of brainstem neurons, including monoaminergic ones, have regenerative capabilities.

316.5 AXONAL REGENERATION IN THE ADULT MAMMALIAN CNS IS PROMOTED BY TRANSPLANTS OF SCHWANN CELLS, CULTURED IN VITRO. Lawrence F. Kromer and Carson J. Cornbrooks. Dept. of Anatomy & Neurobiology, Univ. of Vermont, Burl., VT 05405.

Although CNS axons can grow for considerable distances within peripheral nerve grafts, the complex cellular nature of these grafts has precluded the identification of factors responsible for promoting axonal growth. Thus, the present study was undertaken to develop a transplantation procedure in which restricted cell populations, cultured in vitro, could be used to characterize CNS axonal growth promoting factors that function in vivo.

For these experiments, adult female Sprague-Dawley rats received bilateral aspiration lesions of the fornix, fimbria and supracallosal stria. This procedure denervated the hippocampus (HPC) of its septal cholinergic input and produced a physical gap (2-3mm) between the lesioned surfaces of the septum and HPC, into which the transplants were inserted. The transplants consisted of longitudinal strips of either an acellular collagen substrate or mature preparations of Schwann cells cultured on the collagen substratum for over 2 months. The cultures were prepared from embryonic rat dorsal root ganglia (E15-21) and were treated with antimitotics to remove fibroblasts. The dissected transplants, devoid of neuronal somata and fibroblasts, contained degenerating neurites and myelin, viable Schwann cells and stable extracellular matrix. Acetylcholinesterase (AChE) histochemistry was utilized to identify regenerating septal cholinergic axons within the transplants and host HPC at 1, 6, 14 and 30 days posttransplantation. No AChE-positive axons were observed in association with the acellular collagen substrates or within the host HPC in these specimens at any survival time. In contrast, strands of AChE fibers were observed to enter the septal end of the Schwann cell transplants by day 6. At 14 and 30 days, there was an extremely dense band of AChE fibers localized within the cellular portions but not the collagen substratum of these transplants. AChE fibers also were observed to leave the transplants and reinnervate the anterior end of the host HPC.

These results demonstrate that it is possible to use transplants of selective PNS cell populations, cultured in vitro, to analyze CNS axonal regeneration in vivo. Moreover, the data suggest that Schwann cells and their associated extracellular matrix are responsible for promoting axonal regeneration in the adult mammalian CNS. (Supported by NIH Grant #NS-18126 & the Muscular Dystrophy Association).

316.6 LAMININ AND A SCHWANN CELL SURFACE ANTIGEN PRESENT WITHIN TRANSPLANTS OF CULTURED PNS CELLS CO-LOCALIZE WITH CNS AXONS REGENERATING IN VIVO. C. J. Cornbrooks and L. F. Kromer. Dept. of Anat. & Neurobiol. UVM, Burl., VT 05405.

Macromolecules which reside in the mature peripheral nerve promote axonal outgrowth from regenerating PNS as well as CNS neurons. In order to examine the source and contribution of PNS molecules to the phenomenon of CNS axonal regeneration, methods were devised to transplant cultured PNS cells into intracephalic CNS lesions (see Kromer & Cornbrooks, these abstracts). Antibodies directed against laminin (LAM), a known extracellular matrix (ECM) component, and C4, a cell surface antigen on Schwann cells contacting axons, were used to co-localize regenerating cholinergic CNS axons with molecular components of the transplant. Cryostat sections of brains harvested 1, 6 and 14d posttransplantation were examined from experimental animals hosting cellular transplants containing Schwann cells and ECM on a collagen substratum and lesioned specimens with acellular collagen transplants. In specimens with collagen transplants, LAM+ areas were localized to the brain surface, choroid plexus, ependyma and also appeared to be associated with reactive astrocytes along the cavity. Staining in the cavity quantitatively decreased between 6 and 14d posttransplantation. These specimens exhibited no regeneration of cholinergic axons. Brains hosting cellular transplants contained prominent LAM staining at all time periods in areas identical to those described above, within the cellular regions of the transplant and at the transplant-host interfaces. By 14d, regenerating, cholinergic CNS fibers distinctly co-localized with LAM at the transplant-host interfaces and the cellular portion of the transplant. The C4 antigen was only present on the cellular portion of the transplant at the 1 and 14d time points. Correspondingly, Schwann cells were in contact with PNS axons (albeit degenerating) at 1d and regenerating CNS axons at 14d. In conclusion, immunohistochemical methods reliably identified transplants of PNS cultured cells within intracephalic cavities. Moreover, by 14d posttransplantation, regenerating cholinergic fibers co-localized with LAM+ cells only at the Schwann cell transplant-host interfaces and within the cellular portion of these transplants which contained both ECM (LAM+) and Schwann cells contacting axons (C4+). Therefore, the regeneration of cholinergic CNS axons may not be supported by sources of endogenous CNS LAM and may require neurite promoting factors synthesized by Schwann cells within the transplant. (NIH GRANT #NS-18126 & MDA).

316.7 MOLECULAR CYTOLOGY OF SATELLITE GLIAL CELLS DURING REGENERATION OF MOTONEURONS. G.W. Kreutzberg and M. Gräber*. Max Planck Institute for Psychiatry, D-8033 Martinsried n.Munich, Fed.Rep.of Germany.

In the facial or hypoglossal nuclei resting microglial cells can be stimulated to proliferate by cutting the appropriate motor nerve. Within 3-5 days microglial cells mainly in perineuronal positions divide and cover the surface of the neuronal cell bodies and stem dendrites. This is accompanied by stripping of most of the synaptic boutons normally terminating at these sites (Blinzinger and Kreutzberg, Cell Tiss. Res. 85: 145, 1968). The origin and nature of microglial cells has been debated for some time. They are considered by some to be part of the mononuclear phagocyte system, whereas others consider them to be a third neuroglial element. We have now applied a number of antibodies against various constituents of the cell in order to clarify further the nature of microglia. The cells were clearly shown to have cross reactivity against two different monoclonal and one polyclonal antibody against glial fibrillary acidic protein (GFAP). Anti-S 100 also stained the cells slightly. No decoration has been seen by applying antibodies against fibronectin, laminin, the surface marker L_2, O_4 and C_1. The presence of GFAP-like immunoreactivity in the proliferated satellite microglia lead us to postulate that these cells can be classed as neuroglia, especially belonging to the astrocyte family. However, a reinvestigation of the ultrastructure of the cells has not revealed a type of intermediate filament resembling the typical glial filaments of astrocytes. Microglial cells possess a very dense cytoplasm in which a reticular net of intermediate filaments is present although difficult to recognize.

316.8 FIBROBLAST-LIKE CELLS ACCUMULATE IN THE JUNCTIONAL REGION OF SKELETAL MUSCLES AFTER DENERVATION. Elizabeth A. Connor, Edward Callaway* and U.J. McMahan. Dept of Neurobiology, Stanford University, Stanford, CA 94305.

If the nerves to skeletal muscles are severed, the muscle fibers undergo several striking changes in structure and function. To this list of muscle alterations we add a response that occurs in the connective tissue at or near the former sites of the neuromuscular junctions.

Muscles contain a variety of connective tissue cells including fibroblasts. Fibroblasts have long thin processes with few organelles that course among collagen fibrils. In the frog cutaneous pectoris muscle, which we study, these cells are sparse and evenly distributed throughout the muscle's junctional and extra-junctional regions. We note that following denervation, there is a selective increase in the number of cells resembling fibroblasts in the junctional region. Their processes form a veil around the muscle fibers so that on average more than 95% of the denervated synaptic regions of a myofiber have one or more processes within 1-2 microns of the myofiber membrane. Nuclear counts on cross sections of chronically denervated muscles showed that the junction-specific increase in cell number began within 1 week after denervation and reached a plateau by 3 weeks at which time the number of connective tissue cell nuclei per myofiber was 4 times greater than normal. This increase persisted up to 10 weeks. Throughout this period the number of cell nuclei in extrajunctional regions remained normal. The response was not all or none, since reinnervation of muscles within 1 week after nerve damage, resulted in only a 2-fold increase in the number of cell nuclei.

The source of the fibroblast-like cells is unknown. Unlike the Schwann cells that cap the axon terminals and remain at the synaptic sites after nerve degeneration (Antelman & Connor, in progress), the fibroblast-like cells undergo mitosis as indicated by ^{3}H-thymidine labelling. Thus these cells may be daughters of fibroblasts in the junctional region prior to damage.

Recent studies in this laboratory have revealed that the extracellular matrix of muscles plays an important role in the regeneration of the neuromuscular junctions. Since fibroblasts produce and degrade components of the extracellular matrix, determining the role of the junctional-specific fibroblast-like cell accumulation in the degeneration and regeneration of the neuromuscular junction is of considerable interest.

316.9 THE MAINTENANCE OF NORMAL AND REGENERATING MOTOR NERVE TERMINALS IN THE ABSENCE OF MYOFIBERS. Y.M. Yao & U.J. McMahan. Dept. of Neurobiology, Stanford Univ. Sch. Med., Stanford, California 94305.

The structure of axon terminals at the neuromuscular junction of skeletal muscles is highly ordered and specific. To study the role of the myofiber on the maintenance of axon terminal structure we devised an operation for removing myofibers from the thin cutaneous pectoris (CP) muscle of the frog without damaging axons, axon terminals, the overlying Schwann cells or the synaptic portion of the myofiber basal lamina (BL) sheath. The synaptic sites on the BL, examined by electron microscopy were identified by staining for the enzyme acetylcholinesterase, which is tightly bound to the portion of the myofiber BL in the synaptic cleft and persists after damage to the muscle.

At three months after myofiber removal, the % of synaptic sites on myofiber BL that were occupied by profiles of axon terminals were not different from normal. Between 3 and 6 mo the % of synaptic sites occupied by terminals decreased slightly (10%) and there was a marked increase in the % of axon terminal profiles completely enwrapped by Schwann cell processes. However, at 6 mo the axon terminals, as in normal muscles, contained numerous synaptic vesicles and active zones, and the cross-sectional area of the axon terminal did not differ from control values. Thus, while motor axon terminals of the frog are clearly dependent on the presence of myofibers for maintenance of major structural features, the effects of myofiber absence on these features is gradual and requires months to be detected.

In some experiments we crushed the nerve to the CP muscle 1 mo after removing the myofibers. One month later 55% of the synaptic sites on the BL sheaths were occupied by regenerating axon terminals which contained vesicles and active zones. At 4 mo after myofiber damage, however, only 10% of the synaptic sites were occupied by axon terminals. These findings extend earlier studies in this lab (Sanes et al., J.C.B. 78: 1978) which showed that after removal of myofibers and damage to Schwann cells, regenerating axons did not persist at synaptic sites on empty myofiber BL tubes. We conclude that regenerating axon terminals are far more dependent on the presence of myofibers than normal axon terminals for their maintenance at synaptic sites.

316.10 ROLE OF CELLULAR TERRAIN IN SPINAL CORD REGENERATION.
L. Guth, C. P. Barrett*, E. J. Donati*, and E. Roberts.
Dept. of Anatomy, Univ. of Maryland Sch. of Med., Baltimore, MD 21201 and City of Hope Natl. Med. Center, Duarte, CA.

Recent studies showing that CNS axons will grow into PNS environments indicate that comparable growth into spinal cord lesions could be achieved if the lesion site were populated by astrocytes and ependymal cells rather than by the macrophages, lymphocytes and fibroblasts that generally accumulate at sites of CNS injury. In order to examine this hypothesis experimentally, we performed a laminectomy at T5 and crushed the cord of rats for 1-3 seconds with a smooth forceps (while leaving the dura mater intact to prevent ingrowth of connective tissue). At one week, the lesion was filled with mononuclear cells, degenerating nerve fibers, and capillaries oriented parallel to the long axis of the spinal cord. By two weeks, longitudinally-oriented cords of ependymal cells and astrocytes had migrated into the lesion from the adjacent spinal cord, and similarly-oriented nerve fibers had begun to regenerate into the lesion along the surface of the capillaries, ependymal cells, and astrocytes. The mononuclear cells had now assumed phagocytic activity and were engorged with melin and other cellular debris. After three weeks, the astrocytes had elaborated thick processes within which glial fibers could be discerned by GFAP histochemistry. The regenerated nerve fibers were still oriented longitudinally, but they had increased in number and were often arranged in small fascicles. We conclude that the intrinsic regenerative capacity of spinal cord can be expressed provided ischemic necrosis and collagenous scarring are prevented and the spinal cord parenchyma is first reconstructed by its non-neuronal constitutents.

The reproducibility and non-necrotizing nature of this model of injury renders it suitable for the evaluation of treatments to promote axonal regeneration. After crushing the spinal cord of rats, a polyethylene tube was implanted in such a way that one end lay over the site of the crush injury and the other end was exteriorized at the back of the neck. The dura was then opened and the lesion site superfused with drug or vehicle four times daily for two weeks. The in-vivo ingrowth of CNS axons into the spinal cord lesion was markedly stimulated by treatment with two drugs known to promote in-vitro growth of dorsal root ganglion axons.

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316.11 ELECTRON MICROSCOPY OF HRP INJURY-FILLED REGENERATED AXONS IN THE ADULT FROG SPINAL CORD. F.J. Liuzzi and R.J. Lasek, Dept. of Anatomy, Case Western Reserve Univ., Cleveland, Oh. 44106

Axons of crushed or cut and reanastomosed lumbar dorsal roots regenerate back into the spinal cords of adult frogs and reestablish, in part, the segmental distribution of the root (Liuzzi and Lasek, 1984). Light microscopic examination of cresyl-violet stained transverse sections of lumbar spinal cords after HRP injury-filling of regenerated dorsal roots showed that axons grew mainly along the cord surface in the dorsal funiculus (DF). Some axons penetrated the DF passing ventrally to enter the dorsal gray. However, the majority of regenerated axons entered the gray matter through the dorsolateral fasciculus (DLF). This suggested that there are differences in the DF and the DLF that influence axonal regeneration.

We undertook an EM study to determine the ultrastructural nature of the local environments or domains through which the regenerating axons grow. After survival times of 15-75 days regenerated axons were injury-filled with HRP. The spinal cords were sectioned, processed in DAB, osmicated, run up for EM and flat embedded between two sheets of Aclar plastic for sequential light and EM.

Near the root entry zone the HRP labelled axons coursed longitudinally beneath the pia within the DF as well as in the DLF. EM showed these axons to be associated with the end feet of the glia limitans. Few axons grew through the regions of the DF which were packed with myelin debris and large numbers of oligodendrocytes. Those axons that traversed this gliotic region were associated with the radial processes of astrocytes which resembled embryonic radial glial processes. More axons traversed the DLF, but in this region there was less degenerative debris and the glial processes were more closely packed. These observations suggest that sensory axons regenerating in the adult frog spinal cord preferred to grow along the end feet and radial processes of astrocytes rather than in regions containing myelin debris and oligodendrocytes.

316.12 DEGENERATION AND REGENERATION OF VESTIBULAR AXONS IN AXOLOTL (AMBYSTOMA MEXICANUM) LARVAE. D.A.Covell,Jr.* and P.G.Model. Dept. Neurosci., Albert Einstein Coll. Med., Bronx, NY 10461

In many parts of the nervous system cut nerve fibers can form functional connections with their original target cells and so re-establish their original pattern of connectivity. There are several ways this can be achieved: reclamation of unoccupied postsynaptic sites, displacement of transient terminals from nearby axons, or induction of new sites.

In premetamorphic amphibians and fish, a single pair of identifiable neurons, the Mauthner cells (M-cells), are present in the medulla at the level of entry of the vestibular nerve (nVIII). Axons from the ipsilateral vestibular system provide major sensory input to the M-cell, and in the axolotl, their terminals (club endings) are located in the proximo-ventral surface of the lateral dendrite. To analyze mechanisms operative during axonal regeneration, we severed nVIII of 29-37 mm larvae at the site where the nerve enters the medulla. Alterations in the fine structure of the cut axons were observed as early as 2 hours after surgery: degeneration is initially of the filamentous type and with time, it progresses into the dense type. Pre- and postsynaptic membrane come to be separated from one another by glial processes and later, the glia engulf the terminal remnants. No vacated postsynaptic densities were observed following the separation of club endings from the M-cell surface: the presence of coated vesicles and multivesicular bodies in the M-cell cytoplasm near the degenerating terminals suggests that the M-cell may play an active role in the removal of such densities.

To facilitate identification of nVIII axons during early phases of regeneration as well as following synaptogenesis, horseradish peroxidase (HRP) was put into the vestibular apparatus to label the nerve. Within a week after the lesion, small HRP labeled processes were observed laterally in the medulla in the region normally occupied by the nVIII tract. By 4 weeks, labelled synapses are present on the proximo-ventral surface of the M-cell lateral dendrite. The regenerated fibers form a projection that looks like that of the normal nVIII in that they are largely confined to the same region of the medulla. In addition, they form synapses on the appropriate region of the M-cell.

The data show that cut vestibular axons regenerate and re-establish the original pattern of connectivity. The data suggest that postsynaptic densities are removed when presynaptic terminals degenerate, and thus the new sites may be induced by regenerating axons.

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AXONAL TRANSPORT II

317.1 BIOCHEMICAL ANALYSIS OF HRP RETROGRADE TRANSPORT EFFECT BY AXONAL REACTION AFTER SCIATIC NERVE CRUSH IN CAT. J.C. Liu*, C.F. Chao* and S.D. Wang* Dept. of Biology and Anatomy, NDMC, Taipei, Taiwan Republic of China. (SPON:T.H. Yin)

The morphological and biochemical axonal reactions in neuron cell body after axonal injury have been well established. Axonal transport after nerve injury shows no effect in the rate of orthograde transport, and the total amount of protein transport still remains uncertain. The signal transport for the axonal reaction by nerve injury has been proposed by several authors. The retrograde transport of endogenous or exogenous molecules is one of the possibilities of the signal transport. Our study was initiated to investigate if there is any effect in retrograde transport after nerve injury. HRP was used as the marker in studying the cat sciatic nerve retrograde transport after nerve injury. In this experiment we crushed the peroneal nerve on one side and left the tibial nerve intact and vice versa on the other side at the popliteal region. From 1 to 14 days after the sciatic nerve was crushed, it was sectioned 2cm proximally away from the injured site then immersed in 0.5% of 0.5ml of HRP solution for 2 hours. 24 hours later, both sides of the peroneal and tibial nerves were sectioned into 5mm segments proximally from the terminal end to the gluteal region. Each segment was homogenated and centrifuged in a buffer solution. The supernatents were used for the HRP activity assay and the protein contents were determined by Bio-Rad protein assay. The unit activity of HRP per mg protein for each segment was plotted on the graph. The rate of retrograde transport and the total amount of HRP transport were analyzed. In our results, there are no significant differences in either the rate or the total amount of transport of HRP in the injuried nerve when compared to the control.

317.2 SIZING OF AXONALLY TRANSPORTED VESICLES FROM ADRENERGIC NERVE. D.R. Studelska* and S. Brimijoin. Dept. of Pharmacology, Mayo Medical School, Rochester, MN 55905.

We have attempted to physically characterize and compare the membranous organelles that are the vehicles in which various neuronal components are carried to their destinations along the axon via rapid anterograde and retrograde transport. Because dopamine β-hydroxylase (DBH), a constituent of adrenergic storage vesicles, is subject to rapid bidirectional transport in adrenergic nerves, it was selected as a marker for these intraaxonal structures.

Rat sciatic nerves were ligated in situ for various time intervals. Nerve segments immediately proximal and distal to the point of ligation were subjected to homogenization in an isotonic sucrose buffer to yield DBH-containing particles that had accumulated by anterograde or retrograde axonal transport. Experiments in which the supernatant from an 8,000 g centrifugation of proximal or distal nerve homogenates was subjected to rate centrifugation (150,000 x g for 2 hr) in 5 ml sucrose gradients revealed two populations of sedimenting DBH particles. At 12-24 hr ligation intervals, DBH activity associated with the faster sedimenting particles predominated on the proximal side of the ligation while the pattern of activity was reversed on the distal side. In subsequent experiments, similarly prepared 8,000 x g supernatants were subjected to seiving by Sephacryl S-1000 column chromatography. A comparison of the patterns of eluted enzyme activity revealed that extracts of distal nerve, but not proximal nerve, were dominated by structures large enough to be excluded by the gel (diameters greater than 240 nm). Double separation experiments were performed, in which only the fast or slow sedimenting DBH-containing particle fractions from the sucrose gradients were applied to the S-1000 column. The results showed a nice correspondence between the size and sedimentation rate of particles from proximal nerve. Calibration of the S-1000 column with latex microspheres gave a hydrodynamic diameter of 120 nm for the fast sedimenting particles and 85 nm for the slow sedimenting particles. The results of similar experiments with distal nerve samples were more complex. Heterogenous particle size distributions were found when either slow or fast sedimenting particles were chromatographed. Transmission electron microscopy of these particle fractions is currently underway. (Supported by NIH grant NS 11855.)

317.3 SLOW AXONAL TRANSPORT IN A CNS MOTOR PATHWAY: THE PROTEIN COMPOSITION AND KINETICS OF SCa AND SCb IN HAMSTER CORTICOSPINAL AXONS. M.M. Oblinger, Dept. Biol. Chemistry and Structure, The Chicago Medical Sch., North Chicago IL 60064

Many of the structural and functional properties of axons are dependent on the delivery of cytoskeletal and cytomatrix proteins by slow axonal transport. Because of the essential role of axonal transport in axonal growth, it is possible that aspects of slow axonal transport differ in neurons which differ in their regenerative ability, such as CNS and PNS mammalian neurons. In fact, major differences in the protein composition and rate of both SCa and SCb between peripheral sensory and motor axons and optic axons have been described. However, a lack of definitive information on slow transport in another type of CNS axon prompted the present analysis of axons in a major intrinsic CNS motor pathway.

SCa and SCb proteins in corticospinal axons were radiolabelled by unilateral stereotaxic microinjections of ^{35}S-methionine into the motor cortex of Golden hamsters. Animals were sacrificed 3-50 days later and the brains and spinal cords removed. Frozen sections of brain and cord were serially cut and regions containing corticospinal axons were dissected from each 1 mm section. Rostral to the pyramidal decussation axons contralateral to the injection served as labelling controls; below the decussation the opposite was the case. Axonal samples were solubilized, delipidated and subjected to one and two dimensional SDS-PAGE/fluorography.

Both slow components were identified and characterized in corticospinal axons. SCa moves at 0.2-.5 mm/day and is defined by the neurofilament proteins, tubulin and tau proteins. The SCb component moves significantly faster, contains numerous previously identified polypeptides, but does not contain tubulin. The absence of tubulin in SCb, the 10 fold rate difference between SCa and SCb and several quantitative as well as qualitative differences in slow axonal transport appear to be characteristics that differentiate corticospinal axons (and other CNS axons) from PNS axons that have been studied to date. Such differences in the parameters of protein supply may be important factors in the different capacity for regeneration of these classes of neurons in adult mammals.

317.4 WITHDRAWN

317.5 EVIDENCE THAT ACTIN PARTICIPATES IN THE COMPARTMENTATION OF SEROTONIN, DOPAMINE AND NOREFINEPHRINE IN RAT PC-12 CELLS. D.H. Small* and R.J. Wurtman, Lab. of Neuroendocrine Regulation, M.I.T., Cambridge, MA 02139

Similarities between the events occurring during muscle contraction and those involved in the release of some neurotransmitters, have led investigators to speculate that muscle-like proteins may also be involved in the release of neurotransmitters. Nerve terminals are enriched with contractile proteins such as actin and myosin (Berl et al., 1973, Science 179, 441). We have isolated an actin-like protein from rat brain synaptosomes which binds ^{3}H-serotonin in a specific and saturable manner (K_d=10^{-5}M) (Small and Wurtman, 1984, Proc. Natl. Acad. Sci. USA 81, 959). Dopamine and norepinephrine also compete with ^{3}H-serotonin for binding sites. As there is evidence for the existence of multiple storage pools of neurotransmitters within neurons, it is possible that an actin-bound pool could provide a store of functional neurotransmitter available for release upon membrane depolarization.

In the present study, we examined the possibility that actin might bind serotonin or catecholamines in rat pheochromocytoma (PC-12) cells. PC-12 cells actively take up serotonin and catecholamines, and they have been shown to secrete catecholamines in a Ca^{2+}-dependent manner. When PC-12 cells were incubated with either ^{3}H-serotonin, ^{3}H-dopamine or ^{3}H-norepinephrine (1 μM concentration) a small proportion (approximately 5%) of the radioactivity taken up by the cells was associated with a high molecular weight protein in supernatant fractions isolated from the cell homogenates. The binding to this protein in situ was inhibited by fluoxetine, an inhibitor of the serotonin and catecholamine uptake systems in PC-12 cells. The binding protein could be absorbed onto myosin thick filaments, which bind actin filaments with high specificity. Furthermore, the affinity of the binding protein for myosin was lower in the presence of 1 mM ATP, which also lowers the affinity of myosin for actin. DNase I, which also binds to actin (at a different site) with high affinity and specificity, displaced bound ^{3}H-serotonin from the binding protein. Thus, it is likely that the binding protein is identical to cytoplasmic actin. These results indicate that actin may participate in the compartmention of serotonin, dopamine and norepinephrine within neurons. They further suggest that a Ca^{2+}-dependent mechanism similar to muscle contraction could mediate the Ca^{2+}-dependent release of these neurotransmitters from nerve terminals.

317.6 TRANSPORT FILAMENTS FROM SQUID AXOPLASM CONSIST PRIMARILY OF SINGLE MICROTUBULES. B.J.Schnapp, M.P.Sheetz*, R.D.Vale*, and T.S.Reese. (Spon: E.Nemeth) NINCDS, NIH, at the MBL, Woods Hole, Mass. 02543, and Univ. of Conn., Health Center, Farmington, Conn. 06032.

When axoplasm is extruded from the squid giant axon and placed between two coverslips in an ATP containing buffer having one-half the concentration of salts and amino acids of the normal intracellular compartment, an apparently single class of filaments dissociate from the extruded cytoplasm and settle on the surface of the coverglass not more than 30um away. These filaments support directed organelle movements and are accordingly termed transport filaments (Anat.Rec., 208:157A; Biophysical J., 45: 164A). Electron microscopy of single transport filaments (from dissociated axoplasm prepared by quick-freezing, freeze-drying, and rotary-replication with Pt-Ir) previously identified by video-enhanced light microscopy, indicates that each transport filament is a discrete filamentous structure having a diameter measuring from 22nm to 27nm and a surface substructure consisting of rows of linearly arrayed subunits with a unit spacing up to 5.5 nm within a row and up to 3 nm between rows. This pattern of subunits is indicative of either a single microtubule or a complex of up to 6 actin filaments. To distinguish these possibilities we carried out immunofluorescence using two anti-tubulin monoclonal antibodies, one to the alpha subunit and one to the beta subunit, both raised to chick brain microtubules. For these experiments the dissociated axoplasm was prepared in a Dvorak-Stotler perfusion chamber and the solutions changed by superfusion using a syringe pump. Fixed and rinsed preparations were labelled indirectly using rhodamine-labelled immunoglobulin after exposure to one of the primary antibodies. The anti-alpha tubulin monoclonal labelled greater than 95% of all transport filaments. SDS gel electrophoresis of extruded squid axoplasm and standard immunoblot procedures showed that this antibody labels a single band having a molecular weight of 55,000 daltons and which comigrates with alpha-tubulin from bovine brain. The beta-tubulin antibody failed to label any microtubules in the preparation. Controls were exposed to the secondary antibody only were negative. We conclude that each transport filament consists primarily of a single microtubule. Further experiments are needed to determine whether it is the microtubules themselves or associated components which are directly responsible for organelle movements.

317.7 INDENTIFICATION OF THE MAJOR CALMODULIN-BINDING PROTEINS IN MICROTUBULE PREPARATIONS AS THE SUBUNITS OF A CALCIUM-CALMODULIN-DEPENDENT KINASE. J.R. Goldenring, M.L. Vallano*, R.E. Larson* and R.J. DeLorenzo. Dept of Neurology, Yale Univ. School of Medicine, New Haven, CT 06510.

The ability of calcium-calmodulin to induce the depolymerization of microtubules both in vitro and in vivo is now well established. However, the molecular mechanism for calmodulin's effect on microtubules has remained obscure. Since calmodulin effects are mediated through its interaction with calmodulin binding proteins, we investigated the identity of calmodulin binding proteins in microtubules by the method of Carlin et al. (J. Cell Biol. 87:449, 1981). In thrice-cycled microtubule preparations, only two calmodulin-binding proteins of 52,000 and 63,000 daltons were observed. Both calmodulin-binding proteins showed isoelectric points near neutrality. Phosphocellulose purified tubulin did not display any calmodulin-binding proteins. The calmodulin-binding proteins in microtubules comigrated with the calmodulin-binding subunits of purified calmodulin-dependent kinase (Goldenring et al., J. Biol. Chem. 258:12632, 1983). Calmodulin-dependent kinase activity was observed in microtubule preparations with phosphorylation of alpha and beta tubulin and MAP-2 and two phosphoproteins focusing at neutrality which comigrated with the phosphorylation of the β and α subunits of purified calmodulin-dependent kinase. Two-dimensional tryptic phosphopeptide mapping of the neutral MT phosphoproteins demonstrated identical phosphopeptide patterns to the autophosphorylated subunits of purified calmodulin kinase. In addition, in MT preparations calmodulin-dependent kinase activity phosphorylated alpha tubulin only on serine residues while beta-tubulin phosphorylated 60% on threonine and 40% on serine residues. This pattern is identical to the characteristic phosphorylation pattern observed for the purified calmodulin kinase. All these results indicated the only detectable calmodulin-binding proteins observed in in vitro polymerized microtubule preparations were identical to the subunits of a previously characterized calmodulin-dependent kinase that phosphorylates MAP-2 and tubulin as major substrates. The data suggest that calcium-calmodulin may induce functional effects on microtubule systems through phosphorylation of specific microtubule associated proteins by a calmodulin-dependent protein kinase.

317.8 EVIDENCE THAT GLIAL-NEURONAL TRANSFER OF ^{3}H-PROLINE-LABELED PROTEINS IS A COMPONENT OF THEIR TRANSPORT FROM THE DORSAL COLUMN NUCLEI TO THE INFERIOR OLIVE IN THE CAT. N. Contos and K.J. Berkley. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

When ^{3}H-leucine (^{3}H-leu) or ^{3}H-proline (^{3}H-pro) is injected into the dorsal column nuclei (DCN) of cats, ^{3}H-leu produces dense labeling over neurons and some macroglial cells, whereas ^{3}H-pro produces dense labeling only over macroglial cells (Molinari and Berkley, 1981). Despite ^{3}H-pro's failure to densely label neurons in DCN, light microscopic autoradiography shows that both the internal arcuate fibers projecting from DCN and the terminal regions in the inferior olive (IO) are similarly labeled 24 hrs after either ^{3}H-leu or ^{3}H-pro injections. These findings suggest that ^{3}H-pro-labeled proteins are transported to IO either predominantly through a glial route along the internal arcuate path, or that the ^{3}H-pro-labeled proteins enter DCN's projection fibers by different cellular mechanisms than proteins labeled with other amino acids.

In order to investigate this question, quantitative electron microscope autoradiography was used to specify differences in labeling distribution over samples of internal arcuate fibers from cats whose DCN had been injected with ^{3}H-leu or ^{3}H-pro and allowed to survive 24 hrs. The labeling distribution over ^{3}H-leu samples taken close to the injection site was identical to that over ^{3}H-leu samples taken far from the injection site and close to IO. In ^{3}H-pro samples near the injection site, labeling was most heavily distributed over glial elements, whereas axoplasm accounted for a very small proportion of the labeling. In ^{3}H-pro samples far from the injection site, the labeling distribution approached that of the ^{3}H-leu samples, but was still more heavily distributed over glial elements. Qualitatively, labeling over samples from the inferior olive from both ^{3}H-leu and ^{3}H-pro cases was primarily neuronal (synaptic terminals and axoplasm).

These results demonstrate a shift in the distribution of ^{3}H-pro-labeled proteins from glial to neuronal along the arcuate fiber path from DCN to IO. Such results support the hypothesis that the movement of ^{3}H-pro-labeled proteins from DCN to IO involves a transfer of these proteins from glial cells to neurons where they are then transported to synaptic terminals in IO by axonal transport mechanisms.

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ACTION POTENTIALS AND ION CHANNELS VII

318.1 Ca^{2+}-ACTIVATED Na^+ CURRENT IN PARAMECIUM AND A MUTANT LACKING THIS CURRENT. Yoshiro Saimi* (SPON: M. Epstein). Laboratory of Molecular Biology, University of Wisconsin, Madison, WI 53706.

We have reported the presence of a Ca^{2+}-dependent Na^+ current in Paramecium (Saimi and Kung 1980, J. Exp. Biol.). This current transfers net charge at a rate of ~ 100 pmole/cm^2.sec, much higher than expected of a Ca^{2+}-Na^+ exchange pump. This conductance passes Li^+ and Na^+ but to a much less extent K^+ or any other ion tested and is therefore selective.

Normal paramecia show spontaneous avoiding reactions in Na^+-containing culture medium and the frequency and duration of these reactions increase when the paramecia are presented with a high Na^+ concentration. The complex membrane discharges corresponding to these avoiding reactions have previously been recorded and analyzed. Among the many different types of behavioral mutants of P. tetraurelia, one called fast-2 does not have avoiding reactions when challenged with Na^+.

Voltage-clamp experiments showed that depolarizations fail to induce the Ca^+-activated Na^+ current in fast-2 even when the depolarizing steps were large. To test whether there remains a mutated channel in fast-2 which requires a higher concentration of Ca^{2+} for activation, we used a unique way to inject Ca^{2+} into paramecia using a second mutation, dancer. Dancer fails to properly inactivate its Ca^{2+} channel and allows a large Ca^{2+} buildup in the physiologically relevant compartments as evident by the strong activation of both the Ca^{2+}-activated K^+ current and the Ca^{2+}-activated Na^+ current in dancer (Hinrichsen and Saimi 1984, J. Physiol.). However, the Ca^{2+}-activated Na^+ current remains undetectable in the fast-2/dancer double mutant constructed for this test.

These results will be discussed with respect to the role the Ca^{2+}-activated Na^+ current plays in the complex electric discharges which underlie the most natural and most common type of avoiding reactions of these cells.

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318.2 A SINGLE GENE MUTATION CONVERTS THE RESTING PARAMECIUM MEMBRANE TO A PURE K^+ ELECTRODE. E.A. Richard* and Y. Saimi* (SPON: G. Nicol). Laboratory of Molecular Biology, University of Wisconsin, Madison, WI 53706

The resting membrane potential of Paramecium has been thought to be decided by P_K and P_{Ca} with the resting potential between E_K and E_{Ca}. However in 1 mM Ca^{++} the slope of the resting potential versus $[K^+]_o$ is less than 40 mV per decade and the membrane will not hyperpolarize past -45 mV when $[K^+]_o$ is less than 2 mM. A gamma-ray induced, single locus mutant of P. tetraurelia behaves as a pure K^+ electrode. The mutant membrane continues to hyperpolarize past -100 mV in low $[K^+]_o$ with a slope of approximately 60 mV per decade $[K^+]_o$ change. Similarly an increase in $[Ca^{++}]_o$ above 0.1 mM depolarizes the wild-type membrane with a slope of 16 mV per decade. The membrane potential of the mutant does not respond to changes in $[Ca^{++}]_o$ if $[K^+]_o$ is held constant.

Voltage-clamp experiments and current injection studies showed that most of the depolarization properties of the mutant membrane are intact: the action potential, the transient Ca^{++} current, and the depolarization-induced K^+ current are normal.

Genetic analyses showed that this mutation complements and is not linked to other mutations known to affect the transient Ca^{++} current (pwA, pwB), the Ca^{++}-dependent K^+ current (pant), or the Ca^{++}-dependent Na^+ current (fna).

We will discuss the defect of this mutant with respect to the mechanism which homeostatically adjusts the membrane potential.

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318.3 A STRATEGY FOR ISOLATING MUTANTS WHICH CAUSE OVERPRODUCTION OF THE VOLTAGE-SENSITIVE SODIUM CHANNEL. L.M. Hall and S.D. Wilson*. Dept. of Genetics, Albert Einstein Coll. of Med., Bronx, NY 10461.

A goal of this laboratory is to identify genes involved in the production and regulation of voltage-sensitive sodium channels in excitable cells in Drosophila melanogaster. Previous work from this laboratory involved the characterization of a temperature-sensitive paralytic mutant, no action potential-temperature sensitive (nap^{ts})(Jackson et al., Nature 308:189, 1984). The nap^{ts} mutant has fewer sodium channels than normal as determined by ^{3}H-saxitoxin binding, but the channels which remain appear to have a normal K_D and normal pH sensitivity. In drug feeding experiments using adult flies, we have found that the nap^{ts} mutant is abnormally sensitive to the sodium channel antagonist tetrodotoxin and abnormally resistant to the agonist veratridine. These results can be explained by the fact that this mutant has fewer sodium channels than normal. Since the mutation causes a reduction in the number of functional channels, it is mimicking an antagonist action and therefore should be sensitive to the further antagonistic action of tetrodotoxin. Since the mutant has fewer channels to be activated by the agonist veratridine, it would be expected to be less sensitive to the depolarizing effects of this drug since there would be fewer channels to be activated. Considering the pharmacological phenotype of this sodium channel "underproducer" mutant, we predicted that an "overproducer" mutant should have the reciprocal phenotype. That is, it should be more sensitive to agonists and more resistant to antagonists than wild-type. Thus, we have systematically screened potentially mutant strains for resistance to tetrodotoxin. We have characterized tetrodotoxin-resistant strains by ^{3}H-saxitoxin binding. We report here on the identification of a tetrodotoxin-resistant strain which has 75% more membrane-bound saxitoxin receptors than wild-type. These studies indicate that the number of sodium channels in neuronal membranes can be increased by genetic manipulation. Identification of additional sodium channel overproducers is in progress to determine the number and chromosomal location of genetic loci which can mutate to the overproducer phenotype. This strategy for isolation of sodium channel overproducers should be generally applicable for other neuronal macromolecules for which agonist and antagonist compounds have been identified. (Supported by NIH grant NS 16204. LMH is an Irma T. Hirschl-Monique Weill-Caulier Career Scientist Awardee.)

318.4 CLONING OF POTASSIUM CHANNEL GENE(S) IN THE SHAKER LOCUS OF DROSOPHILA. L.Y. Jan, D.M. Papazian*, Y.N. Jan and P.H. O'Farrell*. Departments of Physiology and Biochemistry, University of California, San Francisco, CA 94143.

Potassium channels are known to control the firing pattern and shape of action potentials, thereby affecting transmitter release. Different potassium channels not only show different voltage sensitivity but also are controlled by different ions, intracellular messengers or transmitter molecules. Thus potassium channels conceivably may control the efficacy of signalling between neurons, as suggested from studies of sensitization in Aplysia (Siegelbaum, Camardo and Kandel, 1982, Nature 299: 413). To understand how the expression and function of potassium channels is regulated in different neurons one needs to characterize these channels in molecular terms. In Drosophila, mutations at the Shaker locus affect the amplitude or the voltage-dependence of a particular potassium channel, the A channel, thereby causing prolonged action potentials and transmitter release (Jan, Jan and Dennis, 1977, Proc. R. Soc. Lond. B. 198: 987; Tanouye, Ferrus and Fujita, 1981, PNAS 78: 6548; Salkoff and Wyman, 1981, Nature 293: 228). Thus studies of the Shaker gene(s) and gene products might lead to molecular studies of potassium channels.

More than 70 kilobases of DNA from the Shaker region has been cloned and analyzed. The restriction map of cloned DNA agrees with the physical map predicted from genomic Southern analyses and reveals that a segment of the Shaker DNA is duplicated once in the Drosophila genome. This segment encompasses three breakpoints in three translocation Shaker mutants and probably contains function relevant to the A channel, as suggested by previous genetic and physiological studies. Currently we are analyzing hybrid dysgenesis-induced Shaker mutants and other Shaker mutants, including Sh^5, the mutant with altered A channel inactivation kinetics. Transformation and transcription studies are also in progress to further define the DNA relevant for potassium channel functions.

318.5 THE GENETICS AND ELECTROPHYSIOLOGY OF SHAKER (Sh) MUTATIONS IN DROSOPHILA MELANOGASTER. L. Iverson*, A. Kamb* and M. Tanouye. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

K^+ channels may largely determine the excitability properties that differentiate one class of neurons from another. Moreover, there is evidence that primitive forms of learning involve modulation of K^+ channel activity. Nevertheless, little is known about the genes that encode K^+ channels, or the gene products themselves.

We have used a combination of genetics and electrophysiology to approach this problem. Sh mutations have been shown to eliminate or modify one type of K^+ current in the fruit fly. We have mapped Sh cytologically, using a collection of Sh translocation stocks, to within 4 bands at 16F on the X chromosome. In addition, a variety of EMS-induced Sh alleles have been mapped genetically with respect to each other, and with respect to nearby recessive lethal mutations and certain translocation breakpoints. It appears that Sh is a complex locus.

We have examined the electrophysiological properties of several Sh alleles in different genetic backgrounds. The K^+ current defect is present in a nerve axon. Various mutant alleles exhibit characteristic action potential waveforms in the presence of different doses of wild-type allele.

318.6 MOLECULAR ANALYSIS OF THE SHAKER (Sh) GENE COMPLEX IN DROSOPHILA MELANOGASTER. A. Kamb*, L. Iverson* and M. Tanouye. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

A variety of genetic and electrophysiological evidence suggests that mutations in the Sh gene complex affect one class of K^+ channels in the fruit fly. Our goal is to characterize the genes responsible for the Sh phenotype. To this end, we have screened a Drosophila genomic DNA library with a cDNA probe known to hybridize within the Sh gene complex and isolated a family of overlapping λ clones from the region. These cloned sequences have been used in turn as probes for a chromosomal walk that ultimately will cover most of the Sh complex.

We have taken two approaches to identify specific mutant loci: first, we have used the Southern blot technique to search for the precise sites of DNA rearrangement in several Sh mutant strains known to contain chromosome translocations within the Sh complex. Some of these breakpoints have been localized and shown to lie within transcription units. Second, we have constructed several P element vectors containing Sh DNA sequences. We are inserting these plasmids into fruit flies, hoping to observe a difference in Sh phenotype of the recombinant flies.

318.7 A DROSOPHILA MUTATION WITH A DEFECT IN Ca^{2+}-DEPENDENT OUTWARD CURRENT. T. T. Elkins*, B. Ganetzky*, and C.-F. Wu. (SPON: C. Woolsey). Dept. of Genetics, Univ. of Wisconsin, Madison, WI. 53706 and Dept. of Zoology, Univ. of Iowa, Iowa City, IA. 52242.

Mutations are being used to probe the molecular identity and function of potassium channels in Drosophila. Conventional two electrode voltage clamp of the dorsal longitudinal flight muscles (DLMs) of adult animals demonstrated the existence of a Ca^{2+} inward current (I_{Ca}), an early transient outward current and a delayed outward current (I_K). The fast outward current has two separate components one of which is voltage dependent (I_A) and the other Ca^{2+}-dependent (I_C) (Salkoff, Nature 302:249, 1983).

We have now discovered a new mutation, slowpoke (slo), that prolongs the depolarization phase of the DLM Ca^{2+} spike from the normal 2 ms. to 30 ms. Voltage clamp analysis of slo DLMs indicates that this mutation greatly reduces I_C but leaves I_A intact. Under voltage clamp conditions, I_A and I_C can be distinguished in normal flies because I_A but not I_C is blocked by 4-AP, whereas I_C but not I_A is eliminated in Ca^{2+}-free Ringer's. Furthermore, I_{Ca} (and therefore I_C) is activated at a more negative voltage than I_A. In contrast, in slo flies I_{Ca} can be activated without eliciting any fast outward current below the voltage at which I_A is activated. In the presence of 4-AP no fast outward current is detectable at all in slo flies. These data indicate that the channels mediating the two components of fast outward current are molecularly distinct, consistent with the observation that Sh mutations eliminate I_A but not I_C (Salkoff, ibid., 1983). The complementary effects of Sh and slo are clearly evident in the DLMs of Sh slo double mutants which entirely lack fast outward currents.

Our results suggest that slo^+ may encode a component of I_C channels and that I_C is the major current responsible for repolarization of the muscle spike. The slo mutation will permit analysis of I_{Ca} uncontaminated by outward currents and may serve as a genetic tool in the study of other Ca^{2+}-dependent processes. It will be of interest eventually to compare at a molecular level the products of the slo locus with those encoded by Sh and eag, two other mutations affecting K^+ currents in Drosophila. (Supported by NIH grants to B. G. and C.-F. W. and a grant from the Searle Scholars Program to C.-F. W.)

318.8 EFFECTS OF SHAKER MUTATIONS ON POTASSIUM CURRENTS IN DROSOPHILA LARVAL MUSCLE. F. N. Haugland* and C.-F. Wu (SPON: R. Wong). Depts. of Physiol. Biophys. and Zoology, Univ. of Iowa, Iowa City, Iowa 52242

Previous studies indicate that mutations in the Shaker (Sh) locus of Drosophila cause increased excitability in both nerve and muscle. Using two micro-electrode voltage clamp, we analyzed kinetic and steady-state properties of membrane currents in identified larval muscle fibers of Sh mutants. In certain mutant alleles (Sh^{KS133}, Sh^{10Z}) the early transient potassium current I_A is eliminated. Examination of the delayed steady potassium current I_K in mutant fibers shows current-voltage (I-V) relations, tail current kinetics, reversal potential and inactivation properties indistinguishable from normal. The alleles which specifically eliminate I_A make possible a detailed examination of I_K in isolation of I_A. The instantaneous I-V relation of I_K shows marked outward rectification. In contrast, this rectification is not present in adult muscles which exhibit large inward I_K tails (Salkoff and Wyman, TINS, 6:128, 1983).

The relation of the Sh locus to the I_A function is most likely complex as evidenced by studies of other alleles that do not eliminate I_A. Although Sh^{tKO120} causes a clear defect in nerve excitability, analysis of I_A in Sh^{tKO120} larval muscle reveals that I-V relations, inactivation and recovery from inactivation are similar to those seen in normal fibers. Moreover, the effects of another allele, Sh^5 on muscle were found to depend on developmental stage. In larval muscle, Sh^5 reduces the amplitude of I_A by shifting the I-V relation to more positive potentials. In contrast, in adult Sh^5 muscles, I_A is normal in amplitude but shows abnormally rapid inactivation (Salkoff and Wyman, 1983). The differential effects of these Sh alleles on excitable membranes support the idea that other genes are involved in the control of I_A (Wu and Ganetzky, Biophys. J., 45:77, 1984) and the possibility that multiple interacting genes participate in the control of various potassium channels. Work supported by NIH grants NS 00675, NS 18500 and a grant from Searle Scholars Program to C.-F. W. F. N. H. supported by NIMH pre-doctoral training grant MH 15172.

318.9 VOLTAGE-DEPENDENT SINGLE-CHANNEL CURRENTS IN DISSOCIATED CNS NEURONS OF DROSOPHILA. Y.-A. Sun* and C.-F. Wu. Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242.

A number of Drosophila mutants are known to affect nerve excitability. However, the normal properties of neuronal membrane currents in this species have not been studied, precluding an analysis of the mutational effects on nerve membranes. Using dissociated CNS neurons from Drosophila larvae, we examined single-channel currents activated by membrane polarizations in cultured type III neurons (Wu et al., J. Neurosci. 3:1888, 1983). Patch electrodes were filled with Drosophila physiological saline to record from cell-attached membrane patches on the soma. Among different classes of single-channel currents activated by membrane depolarization, the most frequently encountered was an outward current resembling the delayed rectifier. It showed a unit conductance of about 7 pS and a mean channel open time in the range of 10-15 ms. The probability of channel opening increased sharply with increasing depolarization. The kinetics of channel opening in response to depolarizing steps indicates that these channels are responsible for a delayed, steady outward current in these neurons. Two other classes of outward current channels have been observed. One type showed a large unit conductance (50 pS) and a long mean channel open time (90 ms). The other class, in contrast, had a briefer channel open time (3-5 ms) and displayed bursting and clustering activities. Inward currents with small amplitude and brief open time were also detected.

In addition, channels activated exclusively by membrane hyperpolarizations are present. Inward currents with properties similar to the inward rectifier reported in other species were recorded, showing a unit conductance of 35 pS and a mean open time of 15-40 ms.

These results indicate that the dissociated CNS neurons retain a variety of functional channels in culture and are thus suitable for analysis of the effects of mutations at a single-channel level. Dissociated neurons from the mutant Sh^{KS133}, which lacks the transient K^+ current I_A in muscles, have been examined. Preliminary results indicate that the frequently encountered channel types described above are all present in this mutant. Further kinetic and pharmacological studies are required to determine the effects of the Sh^{KS133} mutation on the nerve membrane.

Supported by NIH grants NS 00675 and NS 18500 and a grant from the Searle Scholars Program.

318.10 WITHDRAWN

318.PO MUTATIONS OF A GENE AFFECTING POTASSIUM CURRENTS INDUCED BY TRANSPOSABLE ELEMENTS IN DROSOPHILA. B. Ganetzky and C.-F. Wu (SPON: W. I. Welker). Dept. of Genetics, Univ. of Wisconsin, Madison, WI. 53706 and Dept. of Zoology, Univ. of Iowa, Iowa City, IA. 52242.

Molecular studies of the structure and function of potassium channels are restricted by the lack of specific, high-affinity toxins that facilitate biochemical purification. In Drosophila, this problem can be approached by molecular genetic analysis of mutations that alter the properties of potassium channels. One such mutation is eag. Previous voltage-clamp studies of the larval body wall muscle fibers indicated that eag diminished the delayed rectification K^+ current (I_K) (Wu et al. Science 220:1076, 1983). Further analysis of additional eag mutations reveals that the fast transient K^+ current (I_A) may also be affected by particular alleles. Abnormalities in current amplitude and in the instantaneous current-voltage (I-V) relationship have been found. These results suggest that eag^+ plays an important role in the function of two types of potassium channels and possibly encodes a common subunit shared by these channels.

Besides its effect on muscle membrane, eag causes spontaneous repetitive firing of action potentials in motor axons and abnormal release of transmitter at the larval neuromuscular junction. These defects are also attributable to altered K^+ currents and demonstrate the role of the eag^+ in three distinct membrane regions.

To analyze the product of this gene and its role in membrane excitability at the molecular level, attempts to isolate DNA clones are underway. For this, we have screened for mutant eag alleles arising by the insertion of a transposable DNA element (the P factor) into the gene, which provides a molecular tag. Several such mutations have been recovered and the presence of a P factor in the appropriate salivary chromosome region has been confirmed by in situ hybridization. Once the gene has been cloned, it will be possible to correlate the structure of the protein product with its function for both normal and various mutant alleles. (Supported by grants from NIH to B. G. and C.-F. W and a grant from the Chicago Community Trust/Searle Scholars Program to C.-F. W.)

319.1 NEUROCHEMICAL ANALYSIS OF CEREBROSPINAL FLUID IN HUNTINGTON'S DISEASE, R. Kurlan*, R Zazcek*, J. Coyle, I. Shoulson, Dept. of Neurology, Univ. of Rochester, Rochester, NY 14642 and Depts. of Psychiatry and Neuroscience, Johns Hopkins Univ., Baltimore, MD 21205

Measurement of CSF monoamine metabolites and amino acids in Huntington's disease (HD) has yielded conflicting results. We collected CSF from 36 HD patients (Stages I and II, no neuroleptic therapy) under standard activity and diet and from 14 control patients who had other movement disorders. An aliquot from each 5-10 cc CSF sample was analyzed by HPLC-EC for homovanillic acid (HVA) and 5-hydroxy-indole acetic acid (HIAA) and by HPLC-fluorometry using ortho-phthaldehyde derivatization for asparagine (ASN), glutamine (GLN), glutamate (GLU), phenylalanine (PHE) and taurine (TAU). Results from HD and control patients were compared by student t test for pooled data:

	HVA (PMOL/ML)	HIAA (PMOL/ML)	ASN (NM/ML)	GLN (NM/ML)
HD	200.5 ± 82.8 (n = 36)	228 ± 74.5 (n = 36)	6.5 ± 0.99 (n = 35)	514.9 ± 80.2 (n = 35)
Controls	187.8 ± 48.6 (n = 9)	231.1 ± 80.2 (n = 14)	6.84 ± 1.47 (n = 5)	467.3 ± 145 (n = 10)

	GLU (NM/ML)	PHE (NM/ML)	TAU (NM/ML)
HD	5.45 ± 1.11 (n = 17)	8.18 ± 1.51 (n = 35)	53.9 ± 17.4 (n = 32)
Controls	4.31 ± 1.0 (n = 5)	9.46 ± 3.92 (n = 5)	35.3 ± 20 (n = 10)

We found no significant differences in concentrations of HVA, HIAA, ASN, GLN or PHE. CSF glutamate was greater in HD patients and nearly attained statistical significance ($T=2.07$, $p < .052$). Taurine was significantly greater in CSF of HD patients ($T=2.86$, $p < .01$). CSF analyses of additional patients with HD, other neurologic disorders and normal controls are in progress to determine whether the observed differences in taurine and glutamate have diagnostic and pathogenetic significance for HD. (Work supported by USPHS NS 17978 and CRC of University of Rochester).

319.2 **PROPERTIES OF MPTP BINDING IN RAT BRAIN: POSSIBLE ASSSOCIATIONS WITH MONOAMINE OXIDASE.** Bruce Parsons* and Thomas C. Rainbow. Dept. of Pharmacology, University of Pennsylvania Medical School 19104.

N-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) produces parkinsonian symptoms in humans and primates by unknown mechanisms. We have utilized in vitro autoradiography and enzymatic preparations to localize, quantify and characterize MPTP binding sites in the rat.

Our procedures for in vitro quantification of (3H)-MPTP binding sites have been described (Eur. J. Pharm. 98:453). Here, we observed the binding of (3H)-MPTP to frozen rat brain sections to be saturable, specific, reversible and of high affinity. Scatchard analysis indicated a single population of sites (Kd = 15 nM: Bmax = 329 fmol/mg p). LKB Ultrofilm autoradiograms indicated that the highest levels of binding were observed in the arcuate nucleus, dorsal raphe, locus coeruleus and the interpeduncular nucleus (700-1000 fm/mg p). Slightly lower levels were seen within the ependyma of the ventricles and in all circumventricular organs. Moderate levels (100-200 fmol/mg p) were observed in cerebral cortex, central grey and all regions of the hippocampus. Low levels (10-50 fm/mg p) of binding were observed in the caudate-putamen, substantia nigra and the cerebellar cortex. Because the distribution of (3)H-MPTP resembled that of brain MAO (J. Neurochem. 30:263), we plotted the concentration of (3H)-MPTP binding sites against the concentration of MAO for 42 brain structures. A highly significant correlation was obtained ($r = 0.97$; $p < 0.0001$).

MPTP and the MAO inhibitors, clorgyline and Lilly 51514, were the most potent inhibitors of (3H)-MPTP in frozen sections (IC50's approximately 30 nM at 2 nM (3H)-MPTP); deprenyl, pargyline, harmaline and dimethyltryptamine were approximately 1/10 as potent. 5HT and dopamine were approximately 1/500 as potent as MPTP. In vitro enzymatic preparations utilizing (14C)-tryptamine as a substrate for MAO indicated that MPTP inhibited deamination at micromolar concentrations (IC50's: MPTP = 53 µM; clorgyline = 1 µM; deprenyl = 9 µM; pargyline = 33 µM at 6 µM (14C)-tryptamine). Utilization of (3H)-5HT and (14C)-PEA suggested that MPTP was a relatively nonselective inhibitor of MAO (IC 50 = 48 µM for 5HT at 200 µM; 140 µM for PEA at 50 µM).

Our results suggest that MPTP may elicit some of its pathological consequences by inhibiting brain MAO. Such a view is consistent with the hypothesis that MPTP may elevate nigrostriatal dopamine to neurotoxic levels. Supported by NS 19597, Sloan, Klingenstein and Lieberman Fellowships.

319.3 PARKINSONISM-INDUCING TOXIN MPTP: CAN BINDING SITE LOCALIZATION EXPLAIN NEUROTOXICITY? J.A. Javitch, G.R. Uhl and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins University, Sch. of Med., Baltimore, MD 21205.

MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) produces neuropathologic and clinical abnormalities in humans and animals which closely resemble idiopathic Parkinson's disease. The toxin produces loss of nigrostriatal dopamine neurons and neurochemical changes in dopaminergic and non-dopaminergic brain systems in humans, monkeys and mice (Langston, J.W. et al., Science, 219:979, 1983; Burns, R.S. et al., Proc. Natl. Acad. Sci. USA, 80:4546, 1983; Hallman, H. et al., Eur. J. Pharmacol., 97:133, 1984).

We have investigated the binding of radiolabeled MPTP to both rat and human brain. [^{3}H]MPTP binds to a high affinity site in rat brain membranes with a K_D of 28 nM and a B_{max} of 225 pmol/g tissue. In human cerebral cortical membranes, the high affinity site has a similar K_D of 24 nM and a larger B_{max} of 400 pmol/g tissue. Binding is not potently inhibited by a wide variety of drugs or neurotransmitter candidates. The chemical specificity of the binding site corresponds to structure-activity requirements for neurotoxicity. Thus, analogues lacking the N-methyl or the 4-phenyl moieties lack MPTP-like neurotoxicity and are much less potent at the MPTP binding site. The high binding potency (K_i = 41 nM) of the MPTP metabolite, N-methyl-4-phenylpyridine (MPP+), accords with a possible role for this substance in MPTP toxicity.

Autoradiographic studies in human brain show very high binding site densities in the caudate, substantia nigra and locus coereleus, which may explain the neurotoxic and neurochemical sequelae of MPTP administration. In rats, substantia nigra and caudate display only moderate grain densities. This species difference may explain the difficulty in producing selective nigrostriatal degeneration in rats. Sites densely labeled in rat brain include the locus coeruleus, interpenduncular nucleus, arcuate and periventricular hypothalamic nuclei and the subfornical organ. Binding of the toxin to non-nigral areas may cause dysfunction without cell death, allowing these zones to play a role in the expression of the MPTP-induced Parkinsonism.

319.4 IMAGING OPIATE RECEPTORS WITH POSITRON EMISSION TOMOGRAPHY. J.J. Frost*, H.N. Wagner, Jr., R.F. Dannals*, H.T. Ravert*, A.A. Wilson*, J.M. Links*, D.F. Wong*, H.D. Burns*, S.H. Snyder and M.J. Kuhar. (SPON: A.M. Goldberg). The Johns Hopkins Medical Institutions, Balto., MD 21205.

Opiate receptors identified in binding studies mediate the physiological actions of opiate drugs. The 4-carbomethoxy derivatives of fentanyl, such as lofentanil and carfentanil, bind to the mu opiate receptor with high affinity. The K_i of carfentanil for the mu receptor was found to be 0.07 nM in binding studies at 37°C. The K_i's for the delta, kappa and sigma receptors were at least 1000 times higher. Carbon-11 labeled carfentanil (^{11}C-CAR) was synthesized by reacting C-11 methyl iodide with the appropriate carboxylate.

Male ICR mice were injected intravenously with ^{11}C-CAR, sacrificed after 30 min, and the brains rapidly dissected. The thalami, striata, and cerebral cortex are rich in mu receptors while the cerebellum contains a very low receptor level. The thalamus/cerebellum and striatum/cerebellum ratios were 4.1 and 5.2 respectively, calculated as cpm per mg tissue. These ratios correspond to the total/nonspecific binding ratios obtained in in vitro binding studies. Coinjection of 5 mg/kg naloxone reduced the ratios to 1.1 which is consistent with a preferential labeling of opiate receptors in vivo.

Two anesthetized male baboons were imaged with a NeuroECAT scanner after injection of ^{11}C-CAR (about 20 mCi, 620-1200 ci/mmole, 0.3 - 0.5 ug/kg). From 15 to 70 min after injection, preferential accumulation of activity could be seen in the thalami, caudate nuclei and cerebral cortex. Imaging planes were selected by x-ray CT scans. Low levels of activity were found in the cerebellum. At about one hour post injection the maximum caudate, thalami and frontal cortex-to-cerebellum ratios (calculated on per pixel basis) were observed. These values of 4.2, 4.2, and 2.01 respectively, are presumably an underestimate because of the finite spatial resolution of the NeuroECAT. The recovery coefficient for the NeuroECAT is about 0.25, 0.4, 0.7 and 1.0 for the caudate, thalami, frontal cortex and cerebellum and therefore the actual ratios are about 17, 11 and 2.9, respectively. Studies of human subjects are in progress.

These studies demonstrate the feasibility of imaging and measuring opiate receptors in vivo by PET scanning. (Supported by NS15080, MH00053, DA00266, and DA00074).

319.5 IMAGING OF S2 SEROTONIN RECEPTORS IN THE HUMAN CEREBRAL CORTEX WITH IN VIVO POSITRON EMISSION TOMOGRAPHY. H.N. Wagner, Jr.*, D.F. Wong*, R.F. Dannals*, J.J. Frost*, G. Pearlson, H.T. Ravert*, J.M. Links*, M. Titeler and M.J. Kuhar (SPON: W. Jankel). Johns Hopkins Med. Inst., Depts. of Nuclear Medicine, Neuroscience, and Psychiatry, Balto., MD 21205

The imaging by positron emission tomography (PET) of human dopamine receptors in vivo has been reported (Science 221:1264, 1983), and extended to include over 40 normal volunteers and 50 patients with neuropsychiatric disorders. The tracer employed, a neuroleptic, 3-n-methyl spiperone (NMSP), is labeled with a 20 min half-life positron emitter carbon-11 (^{11}C). In vitro binding studies have shown that NMSP, which is similar to spiperone, has a high affinity not only for dopamine D2 receptors, but also for serotonin S2 receptors. The S2 affinity is about 5 fold less.

A variety of pharmacological and regional binding studies indicate that NMSP binds predominantly to S2 receptors in cerebral cortex. For example, cinanserin (an S2 drug) displaces NMSP in cortex with a 100 fold lower dose than does haloperidol (D2 drug). In our human studies using PET we have observed an initial progressive accumulation of ^{11}C NMSP in the frontal, parietal, occipital, and temporal cortical regions, with a plateau and fall off at about 20-30 min after injection, as contrasted with the progressive accumulation in the caudate. This accumulation is intermediate between the D2 caudate binding and the rapid clearing from the cerebellum, where D2 and S2 receptors are absent or in low concentration. This is consistant with known kinetics of NMSP labeling of D2 and S2 receptors.

Studies in 21 normal males have revealed an exponential decline with age in the ratio of frontal cortical to cerebellar activity and a similar curvilinear but lesser fall in 19 normal females. Frontal cortex/white matter ratios showed a similar decline with age. These ratios are estimates of S2 receptor densities and are roughly comparable to ratios of total to nonspecific binding in in vitro studies. Preliminary studies in patients with unipolar and bipolar manic depressive illnesses and with schizophrenia are underway. (Supported by USPHS grants NS15080 and MH00053.

319.6 D2 DOPAMINE RECEPTORS IN NORMAL AND PATHOLOGICAL STATES IMAGED BY POSITRON TOMOGRAPHY. D.F. Wong*, H.N. Wagner, Jr.*, R.F. Dannals*, J.J. Frost*, H.T. Ravert*, J.M. Links*, M. Folstein*, G. Uhl, L. Tune., S. Folstein*, H. Singer*, T. Preziosi* and M.J. Kuhar, (SPON: R.I. Schoenfeld). Depts. of Nuclear Medicine, Neuroscience, Neurology, and Psychiatry, Johns Hopkins Medical Institutions, Balto., MD 21205

We have utilized ^{11}C-n-methylspiperone (^{11}C-NMSP) and positron emission tomography (PET) to label and quantitatively image D2 dopamine receptors in the human brain (Science 221:1264, 1983). NMSP is similar in its binding properties to spiperone, a potent neuroleptic. The initial studies have been expanded to include 21 male and 19 female subjects who ranged in age from 19 to 73 years. D2 receptor binding was estimated by the caudate/cerebellar (Ca/Cb) and the putamen/cerebellar ratios from data taken 43-49 min. post injection.

Accumulation of ^{11}C-NMSP in the receptor rich caudate and putamen increased progressively in time, while radioactivity in the cerebellum, which contains little or no D2 receptors, decreased steadily. Comparison between different individuals revealed a striking decline in D2 binding with age. In males, the data were best fit by an exponential function; Ca/Cb ratio = 2.23 + 7.07 exp (- .06 age); R = .87. There was about a 50% decline in the fitted function over the age range examined. In women there was a smaller decline in binding.

These in vivo data are in agreement with in vitro data obained in postmortem human and rodent tissue in other labs. Loss of D2 receptors on the nigrostriatal terminals and/or loss of striatal dopaminoceptive cells could contribute to the observed changes. Changes in blood flow with age do not seem adequate to explain the decline. These studies show the importance of age and sex matching of controls and patients in clinical studies. They also demonstrate the feasibility of measuring changes in receptors in vivo. Preliminary studies with Huntington's, Parkinson's, schizophrenia and Tourette's patients suggest alterations in receptor populations. Additional studies are in progress. (Supported by NS15080 and MH00053).

319.7 SUBSTANTIA NIGRA RECEPTOR AUTORADIOGRAPHY IN NORMAL, PARKINSONIAN, AND NEUROLEPTIC-TREATED HUMANS. G.O. Hackney*, G.R. Uhl, W.W. Tourtellotte, V.T. Tran, P.J. Whitehouse, J. Javitch, S. Strittmatter, D.L. Price and S.H. Snyder (SPON: R. Kuncl). Depts of Neurology and Neuroscience, Johns Hopkins Medical School Baltimore, MD 21205, Depts. of Neurology and HHMI, MGH, Boston, MA 02114, Wadsworth VA Hospital, Los Angeles, CA 90024.

Nigrostriatal neurons may play important roles in several human movement disorders including Parkinson's disease (PD) and tardive dyskinesia (TD). Definition of receptor populations located in the substantia nigra (SN) and study of SN alterations in these receptors in disease states can help to elucidate normal and pathological influences on these cells. We have used receptor autoradiographic techniques to define nigral drug, neurotransmitter and toxin receptors and to delineate receptor alterations in pathological states.

In normal human SN, high densities of mu opiate (morphine-displacable ^3H-dihydromorphine binding), neurotensin (neurotensin-displacable ^3H-neurotensin binding), somatostatin (SS-14-displacable ^{125}I-LTT-SS28 binding), benzodiazepine I and II (CL218872 and clonazepam-displacable ^3H-flunitrazepam binding) angiotensin-converting enzyme (catopril-displacable ^3H-catopril binding) and nMPTP (N-methyl-4-phenyl-1,2,3,6 tetrahydropyridine-displacable ^3H-nMPTP-binding) sites are found. More modest densities of kappa opiate (U-50,488 displacable ^3H-ethylketocyclozocin), serotonin ($5HT_2$; cinanserin-displacable ^3H-spiperone binding), dopamine (D_2; 2-amino-6,7-dihyroxy-1,2,3,5- tetrahydronapthalene-displacable ^3H-spiperone binding) and cholecystokinin (CCK; CCK-octapeptide-displacable ^{125}I-CCK-32 binding) binding sites are present.

In (PD), binding to mu- and kappa-opiate, neurotensin, CCK and somatostatin receptors is reduced. Benzodiazepine receptor subtypes are differentially affected, with selective depletion of CL-218872-sensitive (type I) binding. There are minimal alterations in serotonin, type II benzodiazepine, and angiotensin-converting enzyme binding.

In neuroleptic-treated schizophrenics, SN neurotensin receptors are markedly elevated with more modest increases in mu-opiate and dopamine receptors.

Study of the differential sequelae of dopamine neuronal loss and dopamine receptor blockade should aid understanding of motor systems abnormalities in PD and TD.

319.8 TRH RECEPTOR BINDING IN SPINAL CORD AFTER EXPERIMENTAL TRAUMATIC AND ISCHEMIC SPINAL INJURY. A. I. Faden, N. S. Pilotte and D. R. Burt. Neurobiology Research Unit, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, and Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD 21201.

Pharmacological treatment with thyrotropin-releasing hormone (TRH) has proved effective in a variety of experimental and clinical disorders involving the motor system, including traumatic spinal and brain injury, motor neuron disease and spinocerebellar degeneration. In contrast, such treatment has proved ineffective following ischemic injury to the brain or spinal cord. Changes in TRH receptor binding have not been evaluated in these conditions. The present studies examined changes in specific binding of ^3H-labeled [3-Me-His^2]-TRH (1 nM, 5 h incubation at 0°C) to membranes of rat or rabbit spinal cord one week following spinal cord injury, using the method of Sharif and Burt (Brain Res. 270:259, 1983). Traumatic spinal cord injury was produced at T-10 in the rat utilizing a modification of the Allen method (75 g-cm impact energy). Ischemic spinal cord injury was produced in rabbits through temporary aortic occlusion (25 min), utilizing a modification of the method of Zivin et al. (Peptides 4:631, 1983). The two forms of injury yielded comparable degrees of hindlimb paralysis at one week after injury. Following traumatic spinal injury in the rat, specific TRH receptor binding was significantly reduced (2.6 ± 0.26 fmol/mg protein), as compared with control animals (4.01 ± 0.22 fmol/mg protein). This decrease was found only at the injury site, not at adjacent lumbar sites or more distant cervical sites. In contrast, ischemic spinal cord injury in the rabbit, which causes severe infarction of ventral gray matter, produced no significant change in TRH receptor binding at the injury site. The present findings indicate that reductions in TRH receptor binding follow only certain classes of spinal cord injury (e.g., traumatic), and that such classes of injury may be those which show beneficial effects from pharmacologically administered TRH.

319.9 DECREASES IN SPINAL RECEPTORS FOR THYROTROPIN-RELEASING HORMONE PRECEDE SYMPTOM DEVELOPMENT IN MURINE LEUKEMIA VIRUS INDUCED MOTOR NEURON DISEASE. D.R. Burt, S.R. Max and P.M. Hoffman*. Depts. Pharmacology and Neurology, U. Md. Sch. Med., Baltimore, MD 21201 and Balt. V.A. Med. Ctr.

Treatment with thyrotropin-releasing hormone (TRH) has been reported to relieve symptoms of amyotrophic lateral sclerosis (ALS) (Engel et al., 1983, Lancet ii:73). The mechanism of this effect is unknown, but known excitatory actions of TRH on surviving motor neurons or trophic actions on neuronal survival may be involved. To investigate the possible role of TRH and its receptors in the pathophysiology of motor neuron disease (MND), we used a model of MND induced by infection with murine leukemia virus (MuLV). Newborn NFS/N mice were injected intracerebrally with Cas-Br-M MuLV and sacrificed at 2, 3, 4, and 5 weeks of age. Surviving mice developed neurological symptoms beginning at 4 to 5 weeks. Spinal cords were removed, chilled and homogenized in cold 20 mM Na-PO_4 buffer. A resuspension of this homogenate was assayed fresh for TRH receptor binding using 1 nM [^{3}H][3-Me-His^2]TRH ([^{3}H]MeTRH) in a single point assay with a 5 h incubation at 0°C. Frozen homogenate was also assayed for muscarinic receptor binding (using [^{3}H]NMS), choline acetyltransferase, and acetylcholinesterase. In two series of experiments, 2 week old MuLV mice displayed a highly significant 20-25% decrease in spinal TRH receptor binding compared to age-matched controls (fmol/mg protein in whole cord: cont. = 11.0 ± 1.4 (4), MuLV = 8.2 ± 0.6 (5), 1st series; cont. = 11.3 ± 0.4 (5), MuLV = 9.0 ± 0.3 (5), 2nd series). This difference was less apparent in 3 week old mice, and had altogether disappeared in MuLV mice at 4 and 5 weeks of age. The decrease in TRH receptor binding thus preceded the onset of clinical neurological signs (4-5 weeks). Pathological changes in motor neurons could not be demonstrated before 3-4 weeks of age. Serum from symptomatic MuLV mice, added in concentrations up to 10%, did not directly inhibit TRH receptor binding to membranes from rat amygdala. No cholinergic marker was significantly different between MuLV mice and controls at any age examined. These results support the possible involvement of TRH or TRH receptors in the pathophysiology of various forms of MND, including ALS. (Supported in part by USPHS (NS20022, MH29671), Vet. Admin., and ALSSOA.)

319.10 ELEVATED CEREBROSPINAL FLUID CONCENTRATIONS OF CORTICOTROPIN-RELEASING FACTOR-LIKE IMMUNOREACTIVITY (CRF-LI) IN MAJOR DEPRESSION. C.B. Nemeroff, E. Widerlöv*, G. Bissette, H. Walleus*, K. Eklund*, I. Karlsson *, P.T. Loosen, C.D. Kilts*, W. Vale. Duke Univ. Med. Ctr., Durham, NC 27710, Univ. Uppsala, Uppsala, Sweden; Univ. Gothenburg, Gothenburg, Sweden; Salk Institute, La Jolla, CA 92138.

It is now widely accepted that alterations in the hypothalamic-pituitary-adrenal (HPA) axis are present in many patients with affective disorder. Hyperactivity of the HPA axis in endogenous depression has been documented by increased serum cortisol and its resistance to suppression after dexamethasone (Carroll, Brit. J. Psychiat. 140:292, 1982). The site within the HPA axis where this endocrine fault(s) resides has not been identified. The recent (Vale et al., Science 213:1344, 1981) elucidation of the structure of CRF and development of a sensitive and specific RIA for this 41-amino acid peptide have now provided the tools for examination of CRF concentrations in CSF of patients with neuropsychiatric disease, including depression. Further impetus for this study was provided by the distribution of CRF-like immunoreactivity (CRF-LI) in the mammalian CNS; high concentrations are contained in limbic (e.g. amygdala) and hypothalamic regions, areas believed to be pathophysiologically altered in affective disorder. CRF was determined in CSF samples by RIA as described in detail elsewhere (Vale et al., Meth. Enzymol. 103:565, 1983) using an antiserum raised in rabbits against rat/human CRF and a tracer of ^{125}I-Tyr°-CRF prepared with chloramine-T and purified by HPLC. CSF samples were collected by lumbar puncture at 9 AM as previously described (Widerlöv et al., Amer. J. Psychiat. 139:1122, 1982) from normal controls (n=10) and drug-free patients with DSM-III diagnoses of major depression (n=23), schizophrenia (n=11), or senile dementia (n=29). The CSF samples were frozen at -70°C, coded and assayed for CRF by two members of the research team ignorant of the diagnostic identity of the samples. The means ± SEM concentrations of CRF-LI (pg/ml) in the groups were as follows: healthy controls = 57.3 ± 3.8; schizophrenics = 56.9 ± 5.6; major depression = 71.8 ± 4.0 and dementia = 56.0 ± 2.4. Both parametric and non-parametric analysis revealed a significant increase in CSF CRF-LI in the major depression group when compared to the controls. No other group differences were noted. These data are consistent with the hypothesis that HPA hyperactivity in major depression is associated with, at least partly, increased CRF secretion.

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319.11 REDUCTIONS OF CEREBROSPINAL FLUID CONCENTRATIONS OF SOMATOSTATIN-LIKE IMMUNOREACTIVITY (SRIF-LI) IN DEMENTIA, MAJOR DEPRESSION AND SCHIZOPHRENIA. G. Bissette, H. Walléus*, E. Widerlöv*, I. Karlsson*, K. Eklund *, P.T. Loosen and C.B. Nemeroff. Duke Univ. Med. Ctr., Durham, NC 27710, USA; Univ. Uppsala, Uppsala, Sweden; Univ. Gothenburg, Gothenburg, Sweden.

Somatostatin (SRIF) is a tetradecapeptide originally isolated from hypothalamus and later localized in other regions of the central nervous system. SRIF inhibits the release of many hormones but more recent research has revealed that this peptide produces a variety of electrophysiological and behavioral effects. The synaptosomal localization of SRIF, its calcium-dependent release from depolarized nervous tissue, the heterogenous CNS distribution of SRIF and its putative receptors at the cellular sites of action of SRIF and the presence of degradative enzymes strongly suggest a neurotransmitter/modulator role for this peptide. There have been several recent studies of somatostatin-like immunoreactivity (SRIF-LI) in the cerebrospinal fluid (CSF) of patients with neuropsychiatric disorders. The concentration of SRIF-LI has been reported to be significantly decreased in dementia, Parkinson's disease, multiple sclerosis and major depression.

Samples of CSF were obtained by lumbar puncture from healthy volunteers, demented patients or patients fulfilling DSM-III criteria for major depression or schizophrenia. Duplicate samples of CSF were lyophilized and assayed for SRIF-LI by radioimmunoassay. Sensitivity of the assay was 2.5 pg/tube. The antisera used recognizes linear SRIF and $SRIF_{1-28}$ as well as cyclic $SRIF_{1-14}$ with equal affinity. The results are as follows (pg/ml SRIF-LI ± SEM): healthy volunteers (n=10), 116.1 ± 15.7; dementia (n=29), 71.3 ± 6.4; major depression (n=23), 67.4 ± 9.2 and schizophrenics (n=10), 60.9 ± 6.8. The demented, depressed and schizophrenic groups all had mean CSF concentrations of SRIF-LI that were significantly lower ($p \leq 0.05$, Student Newman-Keuls Test after ANOVA) than the SRIF-LI concentration in healthy volunteers. Thus, decreases in CSF concentrations of SRIF-LI may reflect impairment of cognitive function and may not be specific for a particular disease entity.

(Supported by NIMH-MH 39415).

319.12 CORTICOTROPIN-RELEASING FACTOR-LIKE IMMUNOREACTIVITY (CRF-LI) IN CEREBROSPINAL FLUID (CSF) IN NORMAL CONTROLS AND MAJOR DEPRESSION: RELATIONSHIP TO CSF MONOAMINE METABOLITES, DST AND TRH STIMULATION TEST RESULTS.

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Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis is one of the most consistent neuroendocrine findings in patients with major depression. This abnormality is reflected in elevated basal plasma concentrations of cortisol or failure to suppress cortisol secretion following dexamethasone administration. CRF was the first hypothalamic releasing factor for which biological activity was detected but it was only recently isolated and sequenced (Vale et al., Science 213:1344, 1981). Serotonin is generally believed to activate, and norepinephrine to inhibit, the HPA axis by an action on CRF secretion. By using a recently developed RIA method for CRF (Vale et al., Meth. Enzymol. 103:565, 1983), CSF concentrations of CRF-LI were determined. The concentrations of CSF monoamine metabolites (MHPG, HVA and 5-HIAA) were determined using a gas chromatography/mass spectroscopy method. CSF samples from healthy volunteers (n=10) and patients with DSM-III diagnosis of major depression (n=23) were collected by lumbar puncture at 9 a.m. Plasma concentrations of TSH at baseline and 30 min. after IV injection of 200 µg TRH were measured after the lumbar puncture. A dexamethasone suppression test (DST) was also performed. No relationships were observed between the CRF values and either basal or post-dexamethasone cortisol concentrations. Two of the 10 controls and 15 of the 23 depressed patients were DST non-suppressors (plasma cortisol concentrations ≥ 5 µg/dl). Six out of 21 depressed patients exhibited a blunted TSH response to TRH. Four of these 6 patients had CSF CRF concentrations greater than the highest normal values. In the healthy volunteers an inverse relationship was observed between CSF concentrations of CRF and MHPG (r=-.72; p=0.019); no relationship was observed between the concentrations of CRF and 5-HIAA or HVA. In contrast, in the depressed population positive correlations were found between CSF concentrations of CRF and 5-HIAA (r=.57; p= 0.004) and between CRF and HVA (r= .43; p=0.039). These data are concordant with the view that norepinephrine and serotonin may be involved in the regulation of CRF secretion. However, these regulatory mechanisms may be altered in patients with major depression.

320.1 EFFECTS OF CLOMIPHENE AND CATECHOLESTROGENS ON DOPAMINE CELL FIRING IN THE RAT SUBSTANTIA NIGRA. W.V. McCall*, T.S. McDowell*, E.H. Ellinwood, Jr., T.H. Lee* and J.K. Nishita. Dept. of Psychiatry, Duke Univ. Med. Center. Durham, NC 27710.

Mild tail pressure (TP) activates various behaviors in the rat that are mediated by the nigrostriatal dopamine (DA) system (Antelman et al., 1975). Application of TP to anesthetized rats has been used to identify two different types of nigrostriatal DA neurons that are responsive to estradiol (E_2): 1) Type A cells increase their firing rates following TP or E_2, and 2) Type B cells decrease their firing rates following TP or E_2 (Chiodo & Caggiula, 1980). We investigated the response of nigrostriatal Type A and B neurons to E_2, 2-hydroxyestradiol, 2-hydroxyesterone, and clomiphene.

Female Sprague-Dawley rats were ovariectomized and tested each week for TP behaviors. Rats were kept in a 12:12 LD-cycle and tested either during the last 6 hr of light or the first 6 hr of dark. All drugs were administered through the jugular vein during chloral hydrate anesthesia. Extracellular recordings were made with tungsten micro-electrodes using the criteria of Guyenet and Aghajanian (1978). Multiple applications of TP were used to verify the reliability ($r = .83$) of the identification of Type A and B neurons. Beta-E_2 (3ng/kg) and the catecholestrogens (3ng/kg) produced a rapid and significant change in cell firing rates ($p<.05$). Both alpha-E_2 (3000 ng/kg) and clomiphene (0.4 mg/kg) failed to produce a significant change in DA-neuron firing. Clomiphene blocked the effect of beta-E_2 (3 ng/kg; $p<.05$), however, alpha-E_2 had no effect on subsequent administration of beta-E_2.

Our data demonstrate that beta-E_2 and its 2-hydroxymetabolites produce similar changes in DA-cell firing rates in the substantia nigra. In addition, we have shown that multiple applications of TP produce reliable changes in cell firing.

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320.2 INVOLVEMENT OF 5-HT RECEPTORS IN THE REGULATION OF BODY TEMPERATURE. G.A. Gudelsky, J.J. Koenig and H.Y. Meltzer. Department of Psychiatry, Univ. of Chicago, and Illinois State Psychiatric Institute, Chicago, IL.

Although the role of 5-HT in thermoregulation is controversial, there is evidence that the systemic administration of serotonergic agents can elicit a hyperthemic response in the rat. Fenfluramine, quipazine and m-chlorophenylpiperazine have been shown to increase rectal temperature in the rat. The present study was undertaken to clarify the nature of the 5-HT mediated hyperthermic response in the rat. The administration of 5-methoxy-N,N-dimethyltryptamine (5MeODMT, 5 mg/kg,ip) and 6-chloro-2-(1-piperazinyl)-pyrazine (MK-212, 1 mg/kg, ip) resulted in increases in rectal temperatures of $0.8 \pm 0.1^{\circ}C$ and $0.6 \pm 0.1^{\circ}C$, respectively. These hyperthermic responses appear to be centrally mediated since the peripheral 5-HT antagonist, xylamidine (5mg/kg), did not significantly alter the increase in rectal temperature produced by 5MeODMT. In addition, 5-methoxytryptamine (10 mg/kg), which does not readily penetrate the blood-brain barrier, did not alter rectal temperature. The selective 5-HT_2 antagonists ketanserin and pirenperone (0.3 mg/kg) both produced significant decreases in rectal temperature. The administration of ketanserin prior to 5MeODMT completely prevented the hyperthemic response and, in fact, resulted in a decrease in rectal temperature much greater than that produced by ketanserin alone. The effects of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a selective 5-HT agonist which binds preferentially to 5-HT_{1A} receptors (Eur. J. Pharmacol., 90, 151) also was examined. 8-OHDPAT (0.05 -0.3 mg/kg) produced a dose-related decrease in body temperature. The hypothermic response to 8-OH-DPAT was partially antagonized by metergoline (1 mg/kg). Interestingly, 8-OH-DPAT-induced hypothermia also was antagonized by spiperone, which has been shown to bind to 5-HT_{1A} sites, but not by ketanserin or haloperidol.

It is concluded that the hyperthermia produced by both 5MeODMT and MK-212 is mediated by central 5-HT_2 receptors. Moreover, it appears that 5-HT_2 receptors are intimately involved in thermoregulation, since blockade of these receptors results in a pronounced hypothermia. In addition, it is tempting to speculate that activation of 5-HT_1 receptors mediates 5-HT-induced hypothermic responses. The opposing nature of 5-HT_2 and 5-HT_1 receptors in thermoregulation may account, in part, for the earlier discordant findings regarding 5-HT and thermoregulation.

320.3 CATALEPSY REQUIRES INTACT CHOLINERGIC FUNCTION, BUT CAN BE CAUSED BY APOMORPHINE. W. R. Klemm, Dept. Vet. Anatomy, Texas A&M University, College Station, TX 77843.

In mice that were scored for the length of time they remained immobile (cataleptic) on an inclined wire grid, I tested two hypotheses: 1) that cholinergic mechanisms need to be intact for full expression of neuroleptic-induced catalepsy, and 2) that dopamine agonists would antagonize catalepsy.

Large doses (80 mg/kg) of the cholinomimetic, pilocarpine, could induce catalepsy. Low doses of pilocarpine caused a pronounced enhancement of the catalepsy that was induced by the dopaminergic blocker, haloperidol. Atropine disrupted haloperidol-induced catalepsy. Intracranial injection of an acetylcholine-synthesis inhibitor, hemicholinium, prevented the catalepsy that was usually induced by haloperidol. These findings suggest the hypothesis that the catalepsy which is produced by neuroleptics such as haloperidol is actually mediated by intrinsic central cholinergic systems. Alternatively, activation of central cholinergic systems could promote catalepsy by suppression of dopaminergic systems. In the tests of dopaminergic reversal of catalepsy, both DA agonists that were tested, apomorphine (4 or 8 mg/kg), and bromocriptine (8mg/kg), were effective in disrupting pilocarpine catalepsy. Bromocriptine had little effect on haloperidol catalepsy and, most surprisingly, apomorphine (4 or 8 mg/kg) actually caused a marked enhancement of catalepsy. When given alone over a range of doses, apomorphine produced catalepsy at the two lower doses. After a low dose of apomorphine (0.3 mg/kg), repeated testing of the same mice showed that catalepsy was most profound at 5 min post injection, with progressive decline thereafter. Thus, apomorphine, but not bromocriptine, can produce catalepsy if the drug is given to an animal under certain conditions of DA receptor blockade or if given in low dose. These results suggest the hypothesis that catalepsy can be differentially mediated by a subclass of dopaminergic receptors.

320.4 THE TOPOGRAPHY OF LOCOMOTION: EFFECTS OF AMPHETAMINE OR SCOPOLAMINE. P.R. Sanberg, M.A. Henault, K. D. Houser, R.M. Krema, G.W. Milliken* and D.A. Johnson. Behav. Neuroscience Laboratory, Dept. of Psychology, Ohio Uni., Athens, OH 45701.

Locomotor activity has proven to be an important measure for elucidating various neurological substrates of behavior. However, many types of brain manipulations produce quantitatively similar changes in locomotion, making it impossible to ascribe specific behavioral deficits to these manipulations. This is in part because only one "activity" variable is typically measured. Activity, per se, involves many aspects of movement, and a multifactorial analysis of these aspects could reveal important qualitative changes that may be related to different underlying mechanisms. The present study examined hyperactivity induced by the dopamine (DA) agonist, d-amphetamine sulfate (AMP) or the acetylcholine (ACh) antagonist, scopolamine hydrobromide (SCO).

Male Sprague-Dawley rats (about 250g) were individually placed into computerized Digiscan Animal Activity Monitors (Omnitech, Inc.), allowed to habituate for 2 hr, and again for 1 hr 3 days later. They were then injected i.p. with either AMP (1 mg/kg), SCO (2 mg/kg) or saline and replaced in the monitor. Twenty-one different locomotor variables were then measured in the horizontal and vertical planes for the next 2 hours. Statistical differences between groups were analyzed using ANCOVA techniques.

The results indicated a profound hyperactivity in both AMP and SCO rats. Although this dose of SCO produced about 50% more activity in the horizontal plane than AMP, vertical (rearing) and stereotypic behavior increased about the same. The topography of horizontal (ambulatory) behavior was also similar between groups; the rats travelled farther, faster and spent more time moving than controls. Differences were reflected by the degree to which various factors contributed to the hyperactivity. Increased speed and movement time contributed to SCO activity, whereas AMP activity reflected mainly an increase in movement time. AMP produced a large increase in clockwise revolutions around the perimeter of the cage.

The similarities in the topography of locomotion produced by drugs acting on the DA or ACh systems support the assumption that the hypothetical DA/ACh balance of movement is mediated by the same neurological substrates. The differences, however, suggest that these substrates may play varying roles in the expression of hyperactivity. Supported by Pratt Family and Friends, OURC, and Tourette Syndrome Association.

320.5 **CACLIUM CHANNEL INHIBITORS DIFFERENTIALLY AFFECT PHENCYCLIDINE- AND AMPHETAMINE-INDUCED BEHAVIORAL STIMULATION IN MICE.** J. Grebb*, K. Ellsworth* and W. Freed* (SPON: P. Oliver). Adult Psychiatry Branch, National Institute of Mental Health, Saint Elizabeths Hospital, Washington, D.C. 20032

Calcium channel inhibitors (CCIs) block phencyclidine (PCP)-induced vasospasm in isolated canine arteries (Altura, B. and Altura, B., Science, 212:1051, 1981) and displace PCP binding in rat brain (Quirion, R. and Pert C., Eur.J. Pharmacol., 83:155, 1982). CCIs also inhibit amphetamine-induced stimulation of catecholamine synthesis in rat striatum (Uretsky, N., et al.: J. Neurochem., 32: 951, 1979) and reduce amphetamine-induced circling behavior in mice with 6-OH-dopamine striatal lesions (Fung, Y. and Uretsky, N., Neuropharmacol., 19:555, 1980). Reviews have emphasized that different CCIs have different effects in different tissues and models. We therefore have investigated the effects of 16 CCIs on PCP- and amphetamine-induced behavioral stimulation in mice. **METHODS:** Swiss-Webster mice were injected IP with either active drug (up to 50 mg/kg of CCI) in vehicle (10% Tween 80 in saline) or vehicle alone. Locomotor activity was measured for 30 min, then 5.0 mg/kg of either PCP or amphetamine was administered IP. The activity during the 30 min following PCP/amphetamine administration was divided by the pre-PCP/amphetamine activity to give stimulation ratios (SRs) (Freed, W., et al., Psychopharmacol., 71: 291, 1980). Data were analyzed by a one-way analysis of variance followed by Scheffe multiple comparisons. The null hypothesis was rejected at the 0.05 level. **RESULTS:** The SR for animals that received vehicle followed by PCP was 2.18 $\pm$ 0.22 (mean $\pm$ SEM). The dihydropyridines had the most marked effect in reducing SRs, e.g., nitrendipine (SR=0.11 $\pm$ 0.04). Flunarizine was also effective (SR=0.59 $\pm$ 0.26). The SR for animals that received vehicle followed by amphetamine was 3.39 $\pm$ 0.25. For the amphetamine-stimulated mice, nifedipine and PY 108-068, reduced the SR; however, other dihydropyridines did not; flunarizine reduced the SR, whereas other piperazines did not. Molsidomine was unique in increasing activity before either PCP or amphetamine was administered. **DISCUSSION:** This study further supports the important observation of differential effects of CCIs within a single model. Use of different CCIs, intracellular calcium inhibitors, and calcium channel enhancers will allow further investigations into the role of calcium in the regulation of behavior and brain function. In addition, reports that CCIs have been useful in patients with mania, dementia, and seizures, suggest that CCIs may have a beneficial role in PCP and/or amphetamine toxicity, as well as in other neuropsychiatric conditions.

320.6 BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF AN ALKYLATING ANALOG OF OXOTREMORINE. C.A. Smith, R.W. Russell*, R.A. Booth*, D.J. Jenden and J. Waite*. Pharmacology Dept., Sch. of Med., Center for Health Sci., Univ. of California, Los Angeles, CA 90024.

Previous work (Ehlert, F.J., Jenden, D.J., Ringdahl, B., Life Sci. 34:985 (1984)) has shown that BM 123 (N-[4-(2-chloroethylmethylamino)-2-butynyl]-2-pyrrolidone) spontaneously cyclizes to form an aziridinium ion (BM 123A) which is a potent and selective muscarinic agonist and binds irreversibly to muscarinic receptors (mAChR). The present series of experiments was designed to study the effects of BM 123 on behavioral and physiological variables known to be sensitive to manipulations of the cholinergic neurotransmitter system. BM 123 was injected into the tail vein of Sprague-Dawley rats in doses of 8, 20 and 50 μmol kg^{-1} at 1 hr intervals. This procedure reduces the mAChR to 10% of normal as judged by [^{3}H-]-QNB binding. In other experiments oxotremorine was injected in a similar series of doses (0.3, 0.75, 1.88 μmol kg^{-1}). Measurements of behavioral and physiological variables began immediately after completion of the injections and continued daily for 26 days. The time course of the changes induced by BM 123 varied widely. Some variables (e.g. tremor, chromodacryorrhea) showed peak changes in < 5 min. and returned to their pretreatment baselines within 5-30 min.; body temperature and nociceptive thresholds showed peak changes of -2.6 °C and + 270% and returned to normal within 6 and 24 hr respectively. Locomotor activity and learned operant responding were impaired for 4 and 8 days. After return to baseline some variables showed a significant rebound in the opposite direction. Only the performance of the fixed interval operant response paralleled the return of the mAChR to their normal levels. All changes elicited by oxotremorine recovered more rapidly than those produced by BM 123, confirming that the latter produces a sustained change in receptor-mediated events which would be expected from an irreversible ligand. These results suggest widely differing sensitivity of different neural circuits to interference by BM 123. The differences could be due to the stabilizing effect of neural feedback loops or to the presence of a large receptor reserve at some sites. (Supported by MH 17691 and DAMD17-83-C-3073).

320.7 ANALGESIA AND CHANGES IN RESIDENTIAL MAZE ACTIVITY PATTERNS PRODUCED BY CLONIDINE INFUSION. B. Culver and E. LaCrosse* Department of Pharmacology and Department of Psychology, Univ. of Wyoming, Laramie, WY 82071.

Clonidine, an alpha-2 adrenergic agonist, has been reported to produce analgesia and certain other opiate-like effects in rats. Studies of locomotor activity in rats administered clonidine have reported conflicting results.

Experiments were designed to study the time course and duration of effects of clonidine infusion in young adult Sprague-Dawley-derived female albino rats (Charles River). Clonidine (2 mg/ml) was administered to twelve rats by s.c. implanted Alzet osmotic minipumps intended to continuously release clonidine at a rate of 2 ug/hr for a period of 7 days.

Experimental and control groups (3 rats/group) were maintained in adjoining residential mazes and studied for 10 consecutive days. The groups were taken out of the mazes for one hour (9 a.m.-10 a.m.) each day at which time analgesic tests were conducted. Locomotor activity over the initial 6 days of infusion was decreased in clonidine rats compared to controls during the first exploratory hour and during the nocturnal period, but clonidine animals were more active than controls during the post-exploratory diurnal period. However, beginning on day 7, the last day of infusion, clonidine rats were also more active than controls during the exploratory and hyperactivity persisted for the next two days. These changes are suggestive of a withdrawal syndrome associated with the discontinuation of chronic clonidine in rats.

Analgesic effects of clonidine infusion were evidenced by significant elevations in response latencies measured in experimental animals tested in both a tail flick (TF) and a paw lick (PL) paradigm. Interestingly, PL & TF latencies were elevated to about the same extent (2- and 5-times, respectively, that of controls) during days of clonidine infusion and the following 4 days. Rats continued to show elevated TF, but not PL, latencies when tested again 1 week and also 2 weeks following clonidine infusion.

In another study morphine (5 mg/kg) was injected on day 5 of infusion to clonidine and control rats 30-45 min prior to testing. Morphine produced an increase in locomotor activity in both clonidine and control groups. Morphine also elevated TF and PL latencies about 4-fold in control animals. However, morphine failed to produce any increase in TF or PL latencies in rats infused with clonidine.

320.8 THE EFFECTS OF LONG-TERM ADMINISTRATION OF PHENYTOIN ON DOPAMINE AND GABA RECEPTOR SENSITIVITY. R. Lalonde*, M.I. Botez* (SPON: Y. Lamarre). Neuropsychology Laboratory, Clinical Research Institute, Montreal, Quebec, Canada H2W 1R7.

Male albino rats (N = 40) were injected with phenytoin (PHT) every day for 20 consecutive days and were tested on days 21 and 28 as to their response to 1 mg/kg of apomorphine, a dopamine receptor agonist. It was found that rats treated with PHT had an increased responsiveness to apomorphine-induced stereotypies on day 28 but not on day 21, which is evidence of dopaminergic supersensitivity after long-term treatment with the drug. These results agree with the hypothesis that PHT is an antidopaminergic agent which produces supersensitivity of dopamine receptors following long-term administration, an effect similar to that of the neuroleptics. The clinical findings of oro-facial dyskinesias produced by PHT in epileptic patients may be explained by these results.

In a second experiment, rats (N = 40) were injected with PHT according to the same design as above and tested as to their response to the GABA receptor agonist, muscimol. At 2 mg/kg muscimol produced a catalepsy response, measured by means of four distinct tests. In the akinesia test, latencies till initiation of movement of the rat in the open field are measured. In the bar test and the grid test, latencies till the displacement of the rat from its initial position are measured. Finally, a bracing reaction test was used, cataleptic rats being more resistant to the horizontal push of the experimenter's hand. Rats treated with PHT showed a decreased responsiveness to muscimol-induced catalepsy on day 21 but not on day 28. Muscimol-induced catalepsy was not antagonized by acute pre-treatment with bicuculline (0.5 mg/kg), a GABA-A receptor antagonist. It is proposed that withdrawal after long-term administration of PHT altered the sensitivity of two neurotransmitter receptors in opposite directions and according to a different time sequence, producing supersensitivity to the dopamine receptor on day 28 and subsensitivity to a GABA receptor not sensitive to bicuculline on day 21.

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320.9 Enhanced sensitivity to naltrexone after chronic administration: Effects of quaternary naltrexone and chlordiazepoxide. P.H. Warren and W.H. Morse*, Laboratory of Psychobiology, Harvard Medical School, Boston, MA.

Under some conditions opioid antagonists, such as naltrexone (NTX), decrease schedule-controlled responding at low doses following a period of chronic administration (supersensitivity). Supersensitivity to the behavioral effects of naltrexone was studied in six food-deprived squirrel monkeys. Responding was maintained under a schedule of food presentation in which ten minute time-out periods alternated with three minute periods, in the presence of a visual stimulus, during which every 30th response was followed by the delivery of a food pellet (FR30). In drug sessions successively greater doses of naltrexone were injected i.m. during each time-out period, and the effects of cumulative doses of the drug on responding were determined in the following FR period. Cumulative naltrexone dose-effect curves were determined during single sessions before and after various regimens of chronic administration of naltrexone. Prior to the chronic treatment a cumulative dose of 10 mg/kg naltrexone was required to decrease responding substantially.

Following daily chronic treatments of 10 or 17.6 mg/kg NTX the cumulative dose-effect curves for naltrexone were shifted to the left of the original curves by approximately one log unit. No supersensitivity was evident when doses of chlordiazepoxide (1 to 10 mg/kg) were given one hour before cumulative doses of naltrexone. Under these conditions the effects of naltrexone were similar to its effects before chronic administration; chlordiazepoxide alone did not affect responding significantly. The effects of quaternary naltrexone, a naltrexone analog with limited access to the central nervous system, were similar to the pre-chronic effects of naltrexone and were not affected by the combined treatments with chlordiazepoxide. These results suggest that the behavioral supersensitivity to naltrexone involves a central component of action and that it can be reversed by a phototypic anti-anxiety drug. (Supported by MH07658, MH14275, RR00168, DA00499, DA02658, MH02094.)

320.10 NEUROCHEMICAL AND FUNCTIONAL CONSEQUENCES AFTER MPTP ADMINISTRATION TO THE RAT M.F. Jarvis*, D. Sugar* and G.C. Wagner. Dept. of Psychology Rutgers University, New Brunswick, NJ 08903

Administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyrine (MPTP) was shown to produce destruction of central dopaminergic cells in humans (Langston et al, Sci. 219 979 1983), rhesus monkeys (Burns et al, Proc. Nat. Acad. Sci. 80 4546 1983) and rodents (Heikkila et al, Fed. Proc. 43 793 1984). The present studies were conducted to investigate the neurochemical and functional consequences following MPTP administration to rats.

Adult male Sprague-Dawley rats were injected SC, twice per day at 12 h intervals for 4 days with 30 mg/kg of MPTP. Control rats received comparable vehicle injections. Rats were allowed a 2 week recovery period and then sacrificed, brains removed and dissected for caudate nucleus. HPLC analysis revealed a significant depletion (43%) of caudate dopamine.

In a second study, rats were first deprived of water for 23.3 h/day and trained in a standard operant chamber to press a lever on a fixed-ratio (FR) 5 schedule for water delivery. Rats were given 4 five min sessions per day with a 20 min intersession interval. When responding was stable, dose-response curves for amphetamine (AMPH) and apomorphine (APO) were established using the cumulative dosing procedure. AMPH and APO were administered IP 5 min before each session (the cumulative AMPH doses were 1, 2, 4, & 8 mg/kg; the APO doses were 0.65, .125, .25, & .5 mg/kg).

Following determination of these 'pre-' dose-response curves, rats were divided into two groups. One group received chronic MPTP as above and the other received control injections. All rats then had a two week recovery period after which baseline responding on the operant schedule was re-established. 'Post-' dose-response curves were then determined for AMPH and APO. It was observed that, with respect to control rats, MPTP-treated rats were tolerant to the disruptive effects of AMPH and supersensitive to the effects of APO.

320.11 USEFULNESS OF CLONIDINE FOR THE TREATMENT OF PREMENSTRUAL TENSION SYNDROME. W.A. Price, A.J. Giannini. Departments of Psychiatry, University of Pittsburgh, Pittsburgh, PA; Northeastern Ohio Universities College of Medicine, Rootstown, OH; Ohio State University, Columbus, OH.

The effectiveness of the alpha-two agonist clonidine (catapress) for treating the symptoms of premenstrual tension syndrome was tested in a double-blind design using twelve human volunteers with moderate to severe symptomatology as determined by the modified Beck-Abraham questionnaire. They received clonidine 0.1 mg qid or placebo qid for thirty days. Concomitant use of psychotropic medication in addition to clonidine was not permitted. Patients were rated using the BPRS (Brief Psychiatric Rating Scale) on the 10th and 25th days of their menstrual cycle prior to starting treatment and then on the 10th and 25th day of clonidine/placebo treatment. Clonidine was superior to placebo in reducing BPRS scores of anxiety, tension, and excitement, but was equally as effective in treating other BPRS scores such as depressive mood, hostility, motor retardation, and blunted affect. Clonidine also had no advantage over placebo in alleviating some of the self-reported physical symptoms that accompany premenstrual tension such as breast tenderness, bloating, and abdominal cramping. Clonidine may, therefore, have clinical utility in the treatment of premenstrual tension syndrome, especially when anxiety, tension, and manic-like excitement are the primary symptoms.

321.1 CHARACTERIZATION OF NEUROEFFECTOR TRANSMISSION TO GUINEA PIG MESENTERIC ARTERIES AND VEINS BY TRANSMURAL AND NERVE TRUNK STIMULATION. O.D. Hottenstein* & D.L. Kreulen (SPON: R.Gruener). Department of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724

Resistance and capacitance vessels differ in functional response to constant sympathetic outflow. These studies examined whether differences in the nature of neuroeffector transmission between arteries and veins may account for their dissimilar sensitivites to nerve activity. Preparations of the inferior mesenteric artery and vein attached to lumbar colonic nerves (LCN) were dissected from male guinea pigs and pinned in a tissue bath for intracellular recording (Krebs,-95%O_2,5%CO_2,37°C). Perivascular electrodes for transmural, field stimulations (TMS) were placed on either side of the vessels about 15 mm from the LCN electrodes for nerve trunk stimulations (NTS). Membrane potentials (Em) were recorded between the TMS electrodes. In artery cells single shock TMS or NTS resulted in excitatory junction potentials (EJPs) which were graded by intensity. When the two stimulations were compared at supramaximal intensities EJPs were greater in amplitude for TMS than for NTS. EJPs were blocked by tetrodotoxin ($3x10^{-7}$M) but not by phentolamine (10^{-6}M). In venous cells single shock NTS produced no EJPs. At low intensity TMS (30-70V) produced no changes in Em but higher intensities (80-150V) resulted in step depolarizations (amplitude: 2-5 mV, duration: 2-10 sec) which were attenuated by prazosin (10^{-6}M). In arteries repetitive nerve stimulations (NS) resulted in slow depolarizations (SDs) which were related to frequency (5-20 Hz) and blocked by prazosin (10^{-6}M). For a given frequency TMS resulted in greater SDs than NTS did. In venous cells NS by either method produced similar SDs which were blocked by prazosin (10^{-6}M). However, in contrast to arterial cells venous SDs occurred at much lower frequencies (0.1-2 Hz). These studies distinguish effects of post-ganglionic nerve impulses on mesenteric arteries and veins in vitro. We propose that functional dissimilarity between mesenteric arteries and veins involves differences in their mechanism of neuroeffector transmission. Supported by AZ Affiliate AHA fellowship, NIH HL27781 & HL01136.

321.2 THE INTESTINAL MUCOSA AS A NEURO-EFFECTOR SYSTEM: CHOLINERGIC AND SEROTONERGIC TRANSMISSION. H.J. Cooke* and H.V. Carey* (SPON: C. Wakefield). Dept. of Physiol., Univ. of Nevada, Reno, NV 89557.

The intestinal mucosa of guinea pig ileum is innervated by neuronal processes whose cell bodies lie within the submucosal plexuses. Electrical field stimulation of intramural neurons in the guinea pig ileum increases chloride secretion that is mediated in part by release of acetylcholine from enteric cholinergic neurons (Cooke, H.J., Am. J. Physiol. 246:G263, 1984). Since electrical stimulation of extrinsic nerves has been shown to increase the release of serotonin from enteric neurons and enterochromaffin cells, we investigated the role of serotonin in the secretory response evoked by electrical field stimulation. Flat sheets of guinea pig ileum with the longitudinal muscle removed were mounted in flux chambers and short-circuit current (Isc), a measure of active ion transport processes, was recorded. Bipolar rectangular stimulus pulses were applied to activate enteric neurons. Serotonin (2.5-100uM) evoked a transient biphasic increase in Isc within 1-2 min of its addition to the serosal bathing solution, and this effect was blocked by removal of chloride from the bathing media or by the serotonin antagonist cisapride (5uM). After tachyphylaxis to 150uM serotonin or in the presence of cisapride, stimulation of enteric neurons evoked a biphasic change in Isc that was similar in magnitude to the response in control tissues. In order to determine whether the mucosal response to serotonin was a direct action on the epithelial cells, or mediated by enteric neurons, tetrodotoxin (0.1uM) was added to the serosal bathing medium. In the presence of tetrodotoxin, the mucosal response to serotonin was reduced by 80%. The change in Isc evoked by addition of serotonin also was reduced by 1uM atropine, and the magnitude of this effect was similar to that in the presence of tetrodotoxin. These results suggest that serotonin does not mediate the mucosal secretory response evoked by stimulation of enteric nerves at epithelial cells; however, serotonin acts at the submucosal ganglia to excite enteric cholinergic motor neurons that influence epithelial ion transport.

321.3 SEROTONIN RECEPTORS ON THE PROCESSES OF INTRINSIC ENTERIC NEURONS: REDUCTION IN THE AGANGLIONIC BOWEL OF ls/ls MICE. T. Branchek, T.P. Rothman and M.D. Gershon, Dept. of Anat. and Cell Biol. Columbia Univ. P&S, New York, NY 10032.

Serotonin (5-HT) has been established as the neurotransmitter of a population of intrinsic enteric neurons that project to both myenteric and submucosal ganglia. In the gut, 5-HT acts on neurons in these ganglia and also on mucosal afferent nerve fibers to trigger the peristaltic reflex. We have recently characterized enteric 5-HT receptors using ^{3}H-5-HT as a radioligand. The receptor was assayed by rapid filtration of isolated membranes and by radioautography. This receptor is different from either of the two 5-HT receptors described in the CNS. Neither the binding of ^{3}H-5-HT nor its physiological actions are affected by classical (types 1 or 2) 5-HT antagonists; however, both are antagonized by the specific dipeptide, N-acetyl-5-hydroxytryptophyl, 5-hydroxytryptophan amide. The enteric receptor has been found by radioautography not only in myenteric ganglia but also at the junction of mucosa and submucosa (M-S). It has been proposed that the M-S site might represent 5-HT receptors on mucosal afferent nerve fibers. Since receptor radioautography does not have adequate resolution to establish which structures are responsible for the M-S labeling, we have radioautographically investigated the distribution of 5-HT receptors in normal and ls/ls mice. The terminal 2 mm of bowel in the ls/ls animals are devoid of intrinsic enteric neurons and processes, although extrinsic autonomic and sensory axons do enter the abnormal gut. 5-HT receptors were found in myenteric ganglia and the M-S region of the entire bowel of normal mice, as well as in the ganglionated proximal bowel of ls/ls mice; however, the M-S labeling by 5-HT was much reduced and myenteric labeling was absent in the aganglionic segments of the ls/ls animals. In addition to the gut, 5-HT receptors were found in the dermis and hypodermis of perianal skin. Silver staining revealed nerve fibers in regions where 5-HT labeling occurred. These results are consistent with the hypotheses that afferent nerve fibers have enteric-type 5-HT receptors and that M-S labeling is greatly reduced in the aganglionic segments of ls/ls mice because intrinsic neurites are absent from this tissue.

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321.4 MODULATION OF THE CHOLINERGIC AND PEPTIDERGIC REGULATION OF GANGLIONIC TYROSINE HYDROXYLASE ACTIVITY. R.E. Zigmond, N.Y. Ip, and C. Baldwin*. Department of Pharmacology, Harvard Medical School, Boston, MA 02115.

We have previously shown that tyrosine hydroxylase (TH) activity in the rat superior cervical ganglion can be acutely increased by nicotinic, muscarinic and certain peptidergic agonists. The neuropeptides which are effective are secretin, vasoactive intestinal peptide (VIP) and PHI. To determine whether there is any interaction between the cholinergic and the peptidergic regulation of TH activity, the effects of secretin and VIP were examined in the presence of a low concentration of carbachol (3 μM). Carbachol potentiated the effect of both secretin (1 nM to 1 μM) and VIP (0.1 μM to 10 μM) at all concentrations of the peptides examined. In order to determine whether this effect of carbachol was mediated by nicotinic or muscarinic receptors, we examined the effects of the nicotinic antagonist hexamethonium and the muscarinic antagonist atropine. It has been hypothesized that, in addition to having postsynaptic cholinergic receptors, ganglia have presynaptic cholinergic receptors which regulate acetylcholine release. Therefore, in order to simplify the analysis of the effect of carbachol, experiments were done with ganglia that had been decentralized four days earlier, in order to cause the preganglionic nerve terminals to degenerate. Carbachol potentiated the effect of secretin in decentralized ganglia as it did in normal ganglia. This potentiation could be blocked by atropine (6 μM) but not by hexamethonium (3 mM). Bethanechol, a selective muscarinic agonist, also potentiated the effects of secretin. These data suggest that the cholinergic potentiation of the peptidergic regulation of TH activity is mediated via muscarinic receptors.

We have previously reported another type of cholinergic-peptidergic interaction in the regulation of TH activity, namely that substance P can completely antagonize the ability of the nicotinic agonist dimethylphenylpiperazinium (DMPP) to stimulate TH activity. The IC_{50} for this effect of substance P is about 3 μM. In order to determine the type of substance P receptor involved in this effect, we examined the ability of two other tachykinins, kassinin and physalaemin, to inhibit this action of DMPP. Neither of these two peptides was effective at any concentration examined (i.e., 0.3, 10 and 30 μM). These results suggest that this action of substance P in the superior cervical ganglion is not mediated by either substance P "type E" or "type P" receptors. Supported by USPHS grants NS 12651 and MH 00162.

321.5 CHANGES IN SMALL INTENSELY FLUORESCENT (SIF) CELL NUMBER AND TYROSINE HYDROXYLASE (TH) ACTIVITY IN SYMPATHETIC GANGLIA OF FISCHER-344 RATS AFTER ADRENALECTOMY. Steven Waller Dept. Physiol. & Pharmacol., Univ. of South Dakota, Vermillion, SD 57069. Pauli Helen* and Stanley I. Rapoport Lab Neurosci., Nat'l Inst. Aging, Bethesda, MD 21205.

Paraganglionic SIF cells have been reported to increase in number in aged rats (Anat. Rec. 201:563) and their secretions are thought to contribute to the elevated plasma catecholamine levels noted.

To determine the effects of removing the major source of plasma catecholamines on SIF cell number and TH activity, male rats of four ages (3, 12, 24, and 30-34 mo) were adrenalectomized. One month later, sympathetic tissues were removed for analyses. Both aging and adrenalectomy were associated with changes in SIF cell containing tissues. For example, in the hypogastric ganglion, both SIF cell number and TH activity were significantly greater in 30-34 mo old rats than in younger rats. Compared to age-matched sham-operated animals, adrenalectomy in 30-34 mo old rats resulted in a significant decrease in the number of SIF cells and increase in TH activity. The reduced number of SIF cells after adrenalectomy suggests that SIF cells may be dependent on a factor secreted by the adrenal gland. To determine if glucocorticoids are responsible, 24 and 30 mo old rats were given hydrocortisone (40 mg/kg, s.c.) daily for two weeks. No significant differences were found between hydrocortisone- and vehicle-treated rats. Similar findings were observed in the celiac-mesenteric ganglion.

The findings of increased TH activity and SIF cell number with age and increased TH activity after adrenalectomy would be consistent with a contribution of SIF cells to plasma catecholamines. The number of SIF cells was reduced after adrenalectomy in aged rats, suggesting a relationship between SIF cells and the adrenal gland. This relationship does not appear to be mediated by glucocorticoids as chronic administration of hydrocortisone did not alter SIF cell number.

321.6 SPINAL INJURY AND SYMPATHETIC ACTIVATION. R.W. Hamill. Monroe Community Hospital/University of Rochester Medical Center, Rochester NY 14603

Previous investigations indicate that spinal injury results in transsynaptic activation of peripheral noradrenergic neurons in adult Sprague-Dawley rats: tyrosine hydroxylase (T-OH) activity, the rate limiting enzyme in catecholamine biosynthesis and a marker of the biochemical adaptability of sympathetic neurons, in the superior cervical ganglia (SCG) is increased 200% of control within 96 hrs of midthoracic spinal injury and this effect is blocked by preganglionic neurectomy. The present studies examine the time course of these effects and whether various components of the sympathoadrenal axis respond similarly to spinal injury. In order to examine the time course, adult rats received spinal transection and SCG T-OH activity examined at 24,48,72, and 96 hrs and 1,2,4,8, and 12 weeks later. Enzyme activity was unchanged 24 hrs, significantly increased ($p < 0.05$) by 48 hrs, peaked at 210% of control by 96 hrs, and remained elevated (150-190% of control) for 2 months before returning to control levels at 3 months. To determine whether other sympathetic ganglia innervated by spinal segments rostral to the lesion are affected similarly, the stellate ganglia (SG) was examined at identical time points. The initial profile of SG enzyme activity overlapped that observed for the SCG: no change at 24 hrs, a significant increase at 48 hrs, and peak activity at 96 hrs. However, the long term profile differed: SG T-OH activity was significantly increased (142% on control; $p < 0.02$) as long as 5 months after paraplegia, the longest time examined. The spectra of sympathetic responses were addressed by examining T-OH activity (nmoles/ganglia) in various ganglia [SCG, SG, sixth lumbar (L-6), and hypogastric ganglia (HG)] and the adrenal gland (AG) 72 hrs following spinal injury (Tx):

	SCG	SG	L-6	HG	AG
Control	4.7 ± .59	6.8 ± .72	1.5 ± .51	6.8 ± .86	88.6 ± 6.6
Tx	8.1 ± .81*	12.3 ± .99*	1.9 ± .19	7.9 ± .86	168 ± 7.2*

(*differs from control at $p < 0.005$)

These studies indicate that profound and prolonged biochemical activation of peripheral sympathetic neurons occur in acute paraplegia and this effect is restricted to ganglia innervated by spinal segments rostral to the lesion and the adrenal medulla. Such alterations may contribute to the autonomic dysfunctions which occur in spinal injury.

321.7 **DECREASED CARDIAC ß-ADRENERGIC RECEPTOR NUMBER IN THE ABSENCE OF CARDIAC HYPERTROPHY IN ZUCKER RATS.** **S. Bass* and S. Ritter (SPON: G. Hegreberg). College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.**

Recently we reported that genetically obese female Zucker rats have reduced cardiac ß receptor concentrations and hypertrophic hearts compared to lean littermates. To assess the relationship between the cardiac hypertrophy and the decreased cardiac ß receptor number in these animals, the rate of weight gain and degree of cardiac hypertrophy was reduced in one group of obese rats by restricting their food intake between 3 and 9 mo of age. Obese and lean littermates were given free access to food during this time. At sacrifice, body weights were 430 ± 6.0g for the calorically restricted obese rats and 686 ± 13.3g and 264 ± 3.9g, respectively, for the obese and lean littermates. Caloric restriction prevented cardiac hypertrophy in the obese rats. Heart weights were 1.097 ± 0.14g, 0.793 ± 0.14g and 0.761 ± 0.02g, respectively, for the obese, the calorically restricted obese, and the lean rats. Cardiac membranes were prepared from whole hearts minus atria and fibrous rings. Using ^{3}H-dihydroalprenolol as the ligand and propranolol as the displacing agent in a standard in vitro binding assay, the density of cardiac ß receptors and the apparent K_D of the radioligand were determined by Scatchard analysis. As reported previously, cardiac ß receptor concentration was reduced in the obese Zuckers, compared to the leans (46.65 ± 2.81 vs 55.84 ± 3.25 fmole/mg protein, respectively $p < .05$). Caloric restriction, rather than reversing the ß receptor deficit, further decreased receptor numbers (38.64 ± 3.43, $p < 0.06$ vs obese and $p < .01$ vs leans). The apparent K_D values for binding did not differ between groups (2.75 ± .021, 2.27 ± 0.32 and 2.80 ± 0.038 nM). Our results show that decreased cardiac ß receptor binding was not reversed in the obese Zuckers by blockade of the cardiac hypertrophy. Therefore, the decreased cardiac ß receptor number apparently is not attributable to cardiac hypertrophy in these rats, but instead may reflect a primary deficit in postsynaptic sympathetic mechanisms.

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321.8 EVIDENCE FOR A DIRECT NEURAL CONTROL OF ADIPOSE TISSUE LIPOPROTEIN LIPASE. N. D. Courtney, T. Kellogg*, D. R. Reed* and S. C. Woods. Department of Psychology, Univ. of Washington, Seattle, WA 98195.

Adipose tissue lipoprotein lipase (AT-LPL) plays a critical role in the storage of triglycerides (TG) in adipose tissue. AT-LPL hydrolyzes TG from circulating lipoproteins, and the released free fatty acids are reesterified and stored as TG inside adipocytes. Little is known of the factors that control AT-LPL activity. We have previously found that AT-LPL activity of retroperitoneal, epididymal or ovarian fat pads is significantly decreased following subdiaphragmatic vagotomy in rats, suggesting a possible neural control over AT-LPL activity. However, all of the fat pads sampled were posterior to the diaphragm such that a direct vs. an indirect (e.g., a change in a circulating factor) effect of vagotomy could not be ascertained. In other studies, autotransplantation (and therefore denervation) of these same fat pads also reduced AT-LPL. The present experiments addressed the question of direct vs. indirect effects by examining a pad anterior to the diaphragm which would presumably not be effected by our vagotomy procedure. In Experiment 1, 30-day-old Long Evans rats underwent subdiaphragmatic vagotomy while a control group was sham operated. Seven weeks later, interscapular white adipose tissue (IS) was removed and processed for AT-LPL. In Experiment 2, IS from one side was autotransplanted under the renal capsule of similar rats. After 7 weeks, it was removed along with intact IS from the contralateral side. No change of IS AT-LPL was observed between vagotomized and non-vagotomized rats in Experiment 1, consistent with a lack of removal of direct innervation. In Experiment 2, AT-LPL activity was significantly reduced in transplanted IS tissue relative to intact tissue (406 ± 59 and 537 ± 53 nmoles FF/min/g, M ± SEM, respectively), showing that when IS is denervated, its AT-LPL activity decreases. Taken together, the results indicate a direct neural control over AT-LPL.

321.9 HYPERPROLACTINEMIA IN COCAINE ABUSE, C.A. Dackis*, M.S. Gold, T.W. Estroff*, D.R. Sweeney, Research Facilities, Fair Oaks Hospital, Summit, N.J. 07901.

It is well established that the secretion of prolactin is under tonic inhibitory regulation by dopamine (DA) (1). Since cocaine acts upon dopamine neurons, we studied plasma prolactin levels in 20 consecutive patients hospitalized for cocaine abuse and lacking dependence on other substances. Each patient had positive serum cocaine levels and none were taking medications. Of the 20 patients (ages 19-34) there were 15 males and 5 females. Plasma prolactin levels were drawn at 8 a.m. within 72 hours of admission and determined in duplicate by radioimmunoassay.

Using the established normal range (10-20 ng/ml) cutoff of 20 ng/ml to define the upper limit of normal, we found that 15 of 20 patients had elevated prolactin levels. The mean and standard deviation of this distribution was 35.5 ± 24.7 ng/ml. Our finding of elevated prolactin in cocaine patients is consistent with an inhibition of DA neurons and subsequent release from tonic DA inhibition of plasma prolactin in chronic cocaine abuse. Although cocaine appears to acutely activate DA neurotransmission by blocking DA reuptake and perhaps releasing DA, chronic cocaine exposure reduces brain DA concentrations (2) and increases postsynaptic DA receptor density (3), which would be consistent with DA inhibition. Prolactin elevations may also result from cocaine-induced serotonergic disruptions. Given the dramatic elevation of prolactin in chronic cocaine abuse, this measure may prove useful in understanding the nature of cocaine-induced neuroendocrine and neurotransmitter disruptions. The duration of hyperprolactinemia after the cessation of cocaine abuse may correspond with "psychological" withdrawal states associated with the abrupt discontinuation of cocaine.

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321.10 ELECTRICAL STIMULATION INCREASES THE PHOSPHORYLATION OF TYROSINE HYDROXYLASE IN THE SUPERIOR CERVICAL GANGLION. Anne L. Cahill* and Robert L. Perlman Dept. of Physiol. and Biophys., U of Ill. Coll. of Med., Chicago, IL 60680

Depolarizing agents, nicotinic and muscarinic agonists, and agents that raise cyclic AMP levels all activate tyrosine hydroxylase (TH) and increase the phosphorylation of this enzyme in the superior cervical ganglion (SCG) of the rat. Because preganglionic stimulation also increases TH activity we have now investigated the effects of preganglionic nerve stimulation on the phosphorylation of TH in the SCG. Ganglia were preincubated for 90 min in medium containing $^{32}P_i$ and then the preganglionic nerve was electrically stimulated. TH was isolated from homogenates of the ganglion by immunoprecipitation followed by SDS gel electrophoresis. ^{32}P-TH was visualized by radioautography and the incorporation of ^{32}P into the enzyme was quantitated by densitometry. Stimulation of the ganglia at 20 Hz for 5 min increased the incorporation of ^{32}P into TH to a level 5 times greater than that found in unstimulated, control ganglia. The magnitude of the increase in TH phosphorylation depended upon the frequency of stimulation. With 20 Hz stimulation, the incorporation of ^{32}P into TH was near maximal within 30 s and remained approximately constant for up to 10 min of continuous stimulation. The increase in ^{32}P incorporation was reversible; within 30 min after cessation of stimulation, the radioactivity incorporated into TH decreased to the level found in unstimulated ganglia. The reversibility of ^{32}P incorporation indicates that stimulation actually increased the phosphate content of TH and did not merely increase the turnover or the specific activity of enzyme-bound phosphate. The nicotinic antagonist hexamethonium inhibited the increase in ^{32}P incorporation by about 50%, while the muscarinic antagonist atropine had no effect. Thus, preganglionic stimulation appeared to increase phosphorylation of TH in part by a nicotinic mechanism and in part by a non-cholinergic mechanism. We have also studied the phosphorylation of TH in homogenates of the SCG. The ganglion contains cyclic AMP-dependent, Ca^{2+}/calmodulin-dependent, and Ca^{2+}/phospholipid-dependent protein kinase activities. TH can be phosphorylated by both the cyclic AMP-dependent and the Ca^{2+}/calmodulin-dependent protein kinases. These kinases may participate in the phosphorylation of TH in response to preganglionic stimulation. (This research was supported in part by grant HL29025 from NIH and by the Earl M. Bane Charitable Trust).

CYCLIC NUCLEOTIDES II

322.1 PARTIALLY PURIFIED STRIATAL ADENYLATE CYCLASE COMPONENTS RETAIN FUNCTIONAL INTERACTIONS. R.E. Gross*, J.Y. Lew*, M. Goldstein and M.H. Makman* (SPON: I.D. Hirschhorn). Depts. of Biochemistry and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, Neurochemistry Laboratories, New York University Medical Center, New York, NY.

The solubilization and initial characterization of rat striatal dopamine (DA) binding sites has recently been reported (J.Y. Lew, M. Goldstein, J. Neurochem., 42(5), 1984). Regulation of the binding of agonists to these sites by guanine nucleotides was retained in a lectin column eluate, suggesting that additional components of the adenylate cyclase (AC) complex are also present. These proteins appeared to exist in an aggregate or micelle form. In this study we investigated the interaction of AC components in this preparation.

Rat striatal homogenates were solubilized with 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO). After centrifugation at 100,000g, the supernatant was applied to a wheat germ agglutinin-agarose (WGA) column, and eluted with N-acetyl glucosamine. AC activity was assayed using a cAMP competitive binding assay.

Mn++ and forskolin potently stimulated AC activity in the partially purified preparation, indicating the presence of the catalytic component. Preincubation with Al^{3+}, Mg^{2+}, and F^- (AMF) stimulated activity up to seven-fold, and cholera toxin was somewhat less effective. It thus appears that the stimulatory component of AC (N_s) is present and interacts with the catalytic component.

Mn^{2+}, forskolin, AMF, and cholera toxin-stimulated AC activity were inhibited by DA from 1-30uM. AMF-stimulation was inhibited by DA and l-epinephrine approximately equipotently, and this was completely blocked by spiroperidol or yohimbine. Although it is not yet clear whether inhibition is mediated through DA or α_2 receptors or both, it is apparent that blockable receptor-mediated inhibition of AC activity is present in this preparation. This suggests the retention of the inhibitory component (N_i) in the WGA column eluate.

Our results support the presence and functional interactions of receptor sites, N_s, N_i, and the catalytic component in the WGA column eluate of a CHAPSO extracted striatal homogenate. Whether this is a special property of the zwitterionic detergent has not yet been determined. (Supported by USPHS grants N-09649, T32 GM7288, NIMH 02717).

322.2 PURIFICATION AND PROPERTIES OF THE β SUBUNIT OF THE GUANINE NUCLEOTIDE BINDING PROTEINS OF ADENYLATE CYCLASE FROM BOVINE BRAIN. R.M. Huff* and E.J. Neer, Dept. of Med., Brigham and Women's Hosp., Harvard Med. Sch., Boston, MA 02115

The stimulatory (N_s) and inhibitory (N_i) guanine nucleotide binding proteins of adenylate cyclase purified from several tissues consist of heterodimers of α and β subunits. The 35,000-36,000 dalton β subunits from N_s and N_i are functionally and structurally indistinguishable. We have purified a 36,000 dalton (36K) protein from bovine brain membranes which interacts with the α subunit of N_i.

The 36K protein was solubilized from membranes in 1% Lubrol-PX and purified by a series of chromatographic steps carried out in buffer containing 0.1% Lubrol-PX and 1 uM GppNHp. The solubilized proteins were passed over DEAE-Sephacel and the peak of N_s activity (measured by reconstitution with resolved brain catalytic unit) was applied to Sepharose 6B. The N_s peak was applied to another DEAE-Sephacel column resulting in a separation of the 36K protein from Ns activity. The 36K protein was further purified by another DEAE-Sephacel column and gel filtration over AcA 44 Ultrogel. The purified protein appears either as a single band or a doublet on SDS polyacrylamide gel electrophoresis. The 36K protein has a Stokes radius of 35 Å determined by gel filtration on calibrated AcA 44 Ultrogel column. Ultracentrifugation in sucrose density gradients prepared in H_2O and D_2O shows that the protein has a $S_{20,w}$ value of 3.2 S and a partial specific volume ($\bar{v}$) of 0.85 cm^3/gm in 0.1% Lubrol-PX indicating that 1.0 gm of detergent is bound per gm of protein. The high value for $\bar{v}$ indicates that the 36K protein is very hydrophobic. The molecular weight of protein and detergent is 83,000 and that of protein alone is 41,000.

This laboratory has shown that the β subunit of N_i enhances the ADP ribosylation of the α subunit of N_i by Islet Activating Protein (IAP) of Bordetella pertussis. The purified 36K protein also increases IAP dependent ADP ribosylation of the α subunit of N_i in a dose dependent manner with a maximal increase occurring at approximately equal amounts of the two polypeptides. Under these conditions the ADP ribosylated α subunit and putative β subunit appear to sediment as a heterodimer. (ACS Grant 380A, and NIH Grants AM 192077 and AM 07337)

322.3 A MONOCLONAL ANTIBODY TO A MEMBRANE COMPONENT THAT INTERACTS WITH BETA-ADRENERGIC RECEPTOR SITE. D. M. Chuang* (SPON: L. deMedinaceli). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

Although beta-adrenergic receptor (BAR) has been highly purified from many sources, its interaction with various membrane components to express its function is far from clear. In an attempt to get a better understanding of these interactions, monoclonal antibodies have been raised using a crude preparation of BAR as the antigen. BAR is solubilized with digitonin from purified plasma membranes of bull frog erythrocytes and then injected into balb/c mice. Spleen cells from immunized mice are further immunized in vitro with the BAR preparation before they are fused with myeloma P3 x 63 Ag8 x 653. Screening for antibody production is made using ELISA and immunoprecipitation of the BAR labeled with ^{125}I-iodohydroxybenzylpindolol (IHYP). Following repeated limiting dilution of the positive hybridoma cultures, monoclonal antibodies are massively produced by ascitic fluid of monoclonal cultures.

One of the monoclonal antibodies, termed DAF, has been extensively characterized. DAF (with a chain composition of Igg_1K) can immunoprecipitate BAR-IHYP complex in the crude preparations of frog erythrocytes, frog heart and C6 glioma cells but fails to immunoprecipitate IHYP complexed to internalized BAR (which is present in the 30,000 x g supernatant derived from frog erythrocytes exposed to isoproterenol for 2 hrs). When solubilized frog BAR is fractionated by a Sephadex G-150 column, two BAR activity peaks are detected; only the peak with a higher molecular weight can be immunoprecipitated. DAF affects neither the specific binding of ^{125}I-IHYP or ^{3}H-dihydroalprenolol to BAR, nor the accumulation of cyclic AMP in intact erythrocytes induced by isoproterenol. However, using purified plasma membranes of frog erythrocytes, DAF has biphasic effects on the isoproterenol-sensitive adenylate cyclase activity. At low concentrations of DAF, isoproterenol-sensitive adenylate cyclase is activated, whereas the activity is inhibited by high concentrations of the immunoglobulin. Immunoblotting of the isoelectrofocusing gel indicates that DAF is bound to a component in the antigen with a pI = 6.2. Direct immunoprecipitation of total erythrocyte proteins labeled with ^{125}I reveals that the immunoglobulin precipitates a major protein with a M. Wt. of roughly 43,000 daltons. Taken together these results suggest that monoclonal antibody DAF is against a membrane component that interacts with BAR and may be essential for BAR to express its function.

322.4 DIFFERENTIAL BLOCKADE OF GTP-DEPENDENT EFFECTS ON THE CHICK CARDIAC MUSCARINIC RECEPTOR-ADENYLATE CYCLASE SYSTEM BY IN VIVO PERTUSSIS TOXIN TREATMENTS. K.K. McMahon*[1], R.D. Green*[2], M.M. Hosey*[1] (SPON: C.M. Combs). [1]Chicago Med. Sch., N. Chicago, IL 60064; [2]Univ. of IL Sch. of Med., Chicago, IL 60680.

Pertussis toxin (0.03 to 1 μg/40 g chick) was injected into newborn chicks and the animals were sacrificed 48 hrs later. Cardiac membrane preparations from control and pertussis toxin treated animals (PTA) were used to study the influence of the toxin on three guanine nucleotide-dependent effects of the muscarinic receptor/adenylate cyclase attenuating system. In control membranes, guanine nucleotides decreased the apparent affinity of the muscarinic receptor for the agonist oxotremorine. The IC_{50} values were 0.1 μM and 0.7 μM in the absence and presence of 100 μM Gpp(NH)p, respectively. In membranes from PTA the IC_{50} values obtained in the absence of Gpp(NH)p increased with the concentration of toxin injected, and the Gpp(NH)p induced increase in IC_{50} values was concomittantly diminished. In membranes from 1 μg pertussis toxin treated animals, the IC_{50} values were 1.2 and 1.6 μM in the absence and presence of Gpp(NH)p. A second guanine nucleotide-dependent effect in this system was on basal adenylate cyclase activity. In control membranes, GTP inhibited basal adenylate cyclase activity in a dose dependent manner. Maximum inhibition was 25% at 10 μM GTP. In PTA membranes, this inhibition was reversed to a GTP dependent stimulation. Basal cyclase in membranes from 1μg toxin treated animals was stimulated by 30% by 10 μM GTP. The third guanine nucleotide-dependent effect was to support the attenuation by oxotremorine of basal or isoproterenol-stimulated adenylate cyclase activity. Unpredictably, the toxin treatments did not alter the potency or efficacy of oxotremorine to cause a GTP-dependent attenuation of basal or isoproterenol-stimulated adenylate cyclase. Toxin induced ADP ribosylation of the membranes was used to determine how complete the in vivo treatment was. A comparison of toxin induced ADP ribosylation of the 39-41 K dalton protein suspected to be the inhibitory coupling protein (N_i) in membranes from control and 1 μg PTA showed that PTA membranes had only 15% of the ^{32}P incorporated from [^{32}P]NAD in that region as did controls. The results suggest there are differential sensitivities of the various functions of N_i in coupling receptor-mediated attenuation of adenylate cyclase. (Supported in part by BRSG #RR-05366, Amer. Heart Assoc. and HL31601.)

322.5 PROPERTIES OF INHIBITED ADENYLATE CYCLASE IN RAT BRAIN MEMBRANES DEPLETED OF STIMULATED ADENYLATE CYCLASE. S.R. Childers, Dept. of Pharmacology, Univ. of Florida Coll. Med., Gainesville, FL 32610.

Neurotransmitters stimulate adenylate cyclase through N_s-, and inhibit the enzyme through N_i-coupling proteins. Because of their heterogeneity, brain membranes have always been a difficult material for assay of inhibited cyclase. Earlier (Childers et al., Life Sci. [1983] 33, 215) we showed that treatment of membranes at pH 4.5 before assay selectively decreases N_s-stimulated cyclase and increases inhibition of cyclase by opiate agonists. We now report other inhibited activities in rat brain membranes.

Rat brain synaptosomal membranes were incubated in pH 4.5 buffer (Na acetate) or pH 7.4 buffer (Tris-HCl) at 0° for 20 min, washed with pH 7.4 buffer and assayed for adenylate cyclase activity in pH 7.4 buffer. In membranes from several regions of rat brain, NaF- and Gpp(NH)p-stimulated activities were inhibited 50-100% by low pH pretreatemnt. In striatal membranes, opioid peptides inhibited activity by 30-50% after low pH pretreatment, with enkephalin analogs most potent, followed by dynorphin peptides, with opiate alkaloids (e.g., morphine) least potent. Opiate-inhibited cyclase was most apparent in striatum, with less inhibition in cortex and hypothalamus, and no activity in cerebellum. These results are consistent with delta (and perhaps kappa) but not mu receptors inhibiting cyclase in striatum.

Carbamylcholine inhibited cyclase in low pH pretreated synaptosomal membranes from both cortex and striatum to a maximum of 30-40% of basal activity. Carbachol inhibition was blocked by atropine but not by nicotinic antagonists. Preliminary evidence suggests that muscarinic-inhibited cyclase is mediated by M_2 receptors.

In low pH pretreated membranes from cortex, somatostatin also inhibited cyclase by 30-50%, with an apparent IC_{50} value of 0.1 μM. In hypothalamus membranes pretreated at low pH, clonidine inhibited cyclase by 25-35%, and its inhibition was blocked by phentolamine and yohimbine but not by beta-adrenergic antagonists. These results indicate that reduction in N_s-stimulated adenylate cyclase increases detection of several classes of N_i-inhibited adenylate cyclase in brain membranes.

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322.6 THE SUBCELLULAR DISTRIBUTIONS OF CALCIUM/CALMODULIN-STIMULATED AND GUANINE NUCLEOTIDE-REGULATED ADENYLATE CYCLASES. S. T. Bissen* and T. Ueda. Department of Pharmacology and Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

The distributions of Ca^{2+}/calmodulin (CaM)-stimulated, guanosine 5'-(β,γ-imino)triphosphate (GppNHp)-stimulated, and GppNHp-inhibited adenylate cyclases were determined in subcellular fractions of bovine cerebral cortex. In the primary and crude mitochondrial subfractions, all the cyclases were associated with the fractions enriched with nerve endings. But further fractionation of the synaptosomal membranes on a sucrose density gradient revealed different patterns of distribution. The GppNHp-stimulated cyclase was primarily associated with the heavier fractions of the gradient. In contrast, the Ca^{2+}/CaM-stimulated and the GppNHp-inhibited cyclases were found in every fraction, but the lighter fractions of the gradient contained predominately these cyclases.

The cyclase that was inhibited by GppNHp was studied in more detail. GppNHp inhibited Ca^{2+}/CaM-stimulated activity approximately 60%, but it also inhibited basal activity and forskolin-stimulated activity 25% to 40% in CaM-depleted membranes. Hence, CaM is not required for the GppNHp-induced inhibition of brain adenylate cyclase activity.

These results indicate that the Ca^{2+}/CaM-stimulated and the GppNHp-inhibited cyclases may have different cellular or subcellular locations than the GppNHp-stimulated cyclase. Also, the differential distributions of the GppNHp-stimulated and -inhibited cyclase systems in subcellular fractions of the brain suggest that these two cyclase systems are not always physically associated.

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322.7 DAILY FLUCTUATIONS IN ADENYLATE CYCLASE ACTIVITY OF THE MALE RAT CORPUS STRIATUM. G.D. Chang* and V.D. Ramirez. Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801

A cAMP radioimmunoassay was used to quantitate cAMP produced in an in vitro adenylate cyclase assay. The incubation medium for the adenylate cyclase assay was made to a final volume of 100 ul containing: 1 mg/ml BSA; 1 or 4 mM ATP; 1 mM dithiotreitol; 0.4 mM EGTA; 8 mM $MgCl_2$; 10 mM theophylline; 10 uM Tris-HCl, pH 7.4; and 10 ug striatal homogenate or 3 ug striatal P2 membranes. Reactions were carried out with 30 min preincubation (with or without test chemical) at 4°C followed by 15 min incubation at 37°C.

Under these assay conditions, adenylate cyclase was capable of responding to both stimulatory (dopamine, norepinephrine and ethanol) and inhibitory agents (haloperidol, enkephalin and acetylcholine). All animals were acclimatized to controlled photoperiod (lights on at 0500 and off at 1900) for at least 10 days prior to experiment. It was found that basal enzyme activity using homogenate in the assay started to increase by 1600 in the afternoon (210 ± 14 pmoles cAMP/mg/min, n=3, experiments, each in triplicates), remained high and reached a peak at 2400 (286 ± 51, n=4). Another peak in enzyme activity was observed at 0800 (324 ± 115, n=6) whereas two troughs occurred at 0300 (191 ± 34, n=4) and between 1000 (173 ± 23, n=6) and 1200 (186 ± 11, n=5). A similar profile of basal activity was also found using P2 membranes in the assay. These changes were in part due to fluctuations in dopamine-sensitive adenylate cyclase activity since responsiveness of the enzyme to 10^{-4} M dopamine displayed a similar pattern.

These results indicate that striatal adenylate cyclase activity of the male rat changes significantly during a 24-hour period, emphasizing the importance of photoperiod in striatal adenylate cyclase activity.

322.8 FORSKOLIN MIMICS SLOW SYNAPTIC EXCITATION IN MYENTERIC NEURONS. D. H. Zafirov*, P. R. Nemeth*, J. M. Palmer* and J. D. Wood. Dept. of Physiology, Sch. of Med., Univ. Nevada, Reno, NV 89557.

Slow synaptic excitation (slow EPSP) in AH/Type 2 myenteric neurons persists for several seconds after transient activation of the synaptic input and the actions of substances that mimic the slow EPSP are prolonged for seconds or minutes after transient application of the substance for periods in the millisecond range. This suggests that activation of an intracellular second messenger might be involved in the slow EPSP. The purpose of the study was to investigate the possibility that long-term synaptic activation of AH/Type 2 myenteric neurons is associated with activation of adenylate cyclase and second messenger function of newly synthesized cyclic nucleotides. Forskolin, which is a potent activator of adenylate cyclase in nerve cells, was used for this purpose. Conventional intracellular methods with 3M KCl-filled microelectrodes were used to record and inject electrical current in AH/Type 2 neurons in guinea-pig small intestine **in vitro**. Forskolin was applied by addition to the superfusion solution (Krebs solution). Neuronal excitability was assessed by counting the number of spikes evoked by intracellular injection of rectangular depolarizing electrical pulses of constant current and duration. A statistically-significant increase in the mean number of spikes evoked per current pulse was interpreted as a reflection of increased neuronal excitability. Based on this criterion, forskolin increased the excitability in all of 24 cells that have been tested in preparations from 20 guinea-pigs with one to three trials per cell. Prior to forskolin, the cells did not discharge spikes to depolarizing pulses or fired only a single spike. After forskolin, the cells fired repetitively throughout the current pulses. The enhanced excitability was associated with membrane depolarization, increased input resistance, suppression of postspike hyperpolarization and spontaneous spike discharge. The threshold concentration was 0.5uM with an exposure time of 30sec, and there was a dose dependent increase in the intensity of the effects between 0.5 and 1.0uM. These effects were reversed by washing with drug-free solution. Reversal of the effects required 5 to 15 minutes of washing when the exposure time was 30sec to 1min. The results implicate activation of adenylate cyclase as a factor in slow synaptic excitation of myenteric neurons and suggests that cyclic nucleotides could be mediators of long-term modulation of excitability in these cells.

322.9 MODULATION OF MULTIPLE POTASSIUM CURRENTS IN DIALYZED BAG CELL NEURONS OF APLYSIA. J.A.Strong* and L.K.Kaczmarek (SPON:S.T.Palayoor) Depts. of Pharmacology and Physiology, Yale Univ. Sch. of Med., New Haven, CT. 06510

In their resting state, the peptidergic bag cell neurons of Aplysia are electrically silent and cannot usually fire repetitively in response to current injections. In response to a brief electrical stimulus to the pleuro-abdominal connective, the cells depolarize and fire repetitively. This afterdischarge (AD) can last for 20 to 60 minutes. There is strong evidence that increases in intracellular cAMP play a role in the initiation of the AD.

We have used the whole-cell patch clamp method to do voltage clamp studies of isolated bag cells in primary culture. The adenylate cyclase activator forskolin (F) (.01-.05 mM) along with theophylline (Th) (1 mM) was used to study the effects of increased cAMP levels on the cells' electrical properties. When the dialysis solutions contained 20 mM EGTA, which eliminated calcium-activated potassium currents, several voltage-dependent outward currents remained. Depolarization from holding potentials more positive than -65 mV revealed two kinetically distinct currents, I-fast and I-slow. Of the two, I-f had a faster rise time during depolarization and faster tail kinetics during repolarization. I-f showed a striking cumulative inactivation with repetitive pulses; an interpulse interval greater than 60 seconds was required to avoid this inactivation. Inactivation of I-f could also be seen during a depolarizing pulse; this inactivation was markedly speeded by F/Th application. In contrast, I-s, which may be analogous to the delayed rectifier seen in most neurons, was reduced in amplitude by F/Th but its kinetics were unaltered.

In addition to these two currents, the cells contain a transient outward current (A-current), seen only after its inactivation is removed at holding potentials below -70 mV. As previously reported (Strong, J.Neurosci, in press), F/Th caused a speeding up of the inactivation kinetics of the A-current. Thus F/Th can affect at least three different currents in these cells. All three effects would tend to make the cells more excitable, and are thus consistent with the electrical changes seen at the onset of AD in the bag cell neurons.

322.10 cAMP AND THE MODULATION OF TRANSMITTER RELEASE AT THE LOBSTER NEUROMUSCULAR JUNCTION. M.F. Goy and E.A. Kravitz. Neurobiology Dept., Harvard Med. School, Boston, MA 02115.

The physiology of the dactyl opener muscle of the lobster walking leg is regulated by a complex control system. In addition to the excitatory and inhibitory transmitters (glutamate and GABA), several neurohormones affect the preparation. One of these neurohormones, serotonin (5HT), acts at low concentrations (threshold=$5x10^{-9}$M) to cause long-lasting changes in muscle and nerve: postsynaptically 5HT induces a sustained calcium-dependent contracture in the muscle without causing significant change in membrane potential, and presynaptically it increases evoked transmitter release from both excitatory and inhibitory terminals. The effect on transmitter release from excitatory nerve terminals consists of two distinct components: when 5HT is washed out of the preparation, the decay of the enhanced synaptic response can be approximated as the sum of two exponentials, with time constants of 9 and 125 min (T=13° C). Pharmacological experiments suggest that these two components are mediated by different types of 5HT receptors.

In this preparation, as in many other systems, 5HT causes an increase in tissue levels of cAMP. We have investigated the physiological role of cAMP by using agents that mimic, potentiate, or inhibit the ability of 5HT to alter intracellular levels of this nucleotide. These experiments suggest that cAMP is not directly involved in the mechanism by which 5HT induces tension in muscle fibers: phosphodiesterase inhibitors, such as IBMX (0.5mM) or SQ20,009 (0.2mM), and adenylate cyclase activators, such as forskolin (0.03mM), neither cause significant contraction by themselves nor potentiate the ability of 5HT to cause contractures. Furthermore, long-term exposure to 1mM 8-Br-cAMP fails to increase muscle tension. In contrast, cAMP does appear to play a role in enhancing transmitter release. However, of the two components of 5HT's presynaptic action, only the slower-decaying phase appears to be dependent on the cyclic nucleotide. This phase is selectively potentiated by forskolin and by phosphodiesterase inhibitors; furthermore, phosphodiesterase inhibitors greatly decrease the rate of decay of this phase, without affecting the early phase. Thus it appears that 5HT can enhance transmitter release at the lobster neuromuscular junction by two mechanisms, one cAMP-dependent and the other cAMP-independent. (Supported by NIH grant #NS 07848).

322.11 PEPTIDERGIC NEURONS OF APLYSIA LOSE THEIR RESPONSE TO CYCLIC AMP DURING A PROLONGED REFRACTORY PERIOD. J.A.Kauer and L.K.Kaczmarek. Depts. of Pharmacol. and Physiol., Yale Univ. Sch. of Med., New Haven, CT 06510.

Although the peptidergic bag cell neurons of Aplysia are ordinarily silent, brief electrical stimulation elicits an afterdischarge (AD) lasting approximately 30 minutes. Following this AD the cells enter a refractory period lasting about 18 hours, during which stimulation triggers only brief ADs or fails to trigger an AD. The onset of the first long lasting AD is linked to an elevation of cAMP levels in the bag cell neurons. We have now found that levels of cAMP are elevated with stimulation in the refractory period whether or not a second AD occurs. The elevation was similar to that seen with stimulation of a 1st AD (pmols cAMP/mg protein: control, 9.6±0.9; stim. of 1st AD, 13.7±1.9; stim. in refractory period, 16.5±1.5) (methods,see Kaczmarek,et al.,PNAS 75:5200,1978).

The electrophysiological response of the bag cell neurons to elevating cAMP levels pharmacologically was examined at three time points: 1) before the stimulation of a first AD, 2) 10 min. after the end of a 1st AD, and 3) in the refractory period 1 hour after the end of a 1st AD. Treatment of the bag cell neurons with the adenyl cyclase activator forskolin (F)(50 μM) in the presence of theophylline (T)(1mM) prior to stimulation significantly prolonged the duration of the 1st AD (minutes: control, 32.3±2.7; FT, 64.5±22.1). Treatment with FT within 10 minutes of the end of a 1st AD also significantly prolonged the duration of 2nd ADs stimulated at this time (2nd AD, min.: control, 3.2±2.1; FT, 20.0±5.9). When, however, FT was added 60 minutes after the end of the AD, durations were identical to those obtained without FT (2nd AD, min.: control, 4.0±2.2; FT 2.8±1.3). Furthermore, when FT was added before the 1st AD or at 10 minutes following the 1st AD, the bag cell neurons would often afterdischarge spontaneously. This never occurred when FT was added 60 minutes following the 1st AD. Measurements of cAMP levels in the presence of FT showed that the lack of effect of FT in the refractory period was not due to failure to elevate cAMP levels at this time.

Our results suggest that at least one component of the electrophysiological response to cAMP in these neurons is attenuated or lost after the onset of the refractory period.

OPIATE EFFECTS ON BEHAVIOR

323.1 VARIABLES AFFECTING TREATMENT OUTCOME IN MIDDLE AND UPPER CLASS OPIOID AND COCAINE ABUSERS AND ADDICTS. D.M.Ockert[1]*, I. Extein[2], and M.S. Gold[3]. [1] Columbia University, N.Y.,N.Y. 10025, [2] Falkirk Hospital, Central Valley, N.Y. 10917, [3]Fair Oaks Hospital, Summit, N.J. 07901.

We studied the treatment outcome of a multimodality treatment program whose patients included predominantly middle and upper socioeconomic class drug abusers and addicts. The majority of the drug abuse literature deals with lower socioeconomic status patients. This research, therefore, is unique in studying a significantly different population. Subjects consisted of 101 consecutive voluntary inpatient opioid and cocaine admissions to Fair Oaks Hospital, a private psychiatric hospital in Summit, NJ. The average patient was 29, male, white, Catholic, employed, with 2 years of college, earning $45,00 yearly, and addicted for 7 years. Patients consisted of 25 addicted to heroin, 22 methadone, 19 mixed opioids, 13 cocaine and 22 speedballs (heroin plus cocaine). The treatment program included clonidine detoxification if needed, psychiatric evaluation and treatment, including psychotropic medication if indicated, and outpatient aftercare emphasizing abstinence. Patients were rated using the Addiction Severity Index on admission and 6 months later (an average of 3.5 months after hospital discharge).Ratings were performed by an independent investigator not involved in treatment. Of the 101 patients, 74 completed the inpatient program of which 51 entered the outpatient aftercare program. At followup, 47% of the original patients were drug free. Methadone addicts had the highest readdiction rate followed by heroin, speedballs and cocaine. Cocaine abusers were significantly more likely to be drug free than opioid addicts. Successful completion of the inpatient program, entry into the outpatient program, outpatient length of stay, and presence of social supports all were associated with drug free outcome. The 25 patients treated with psychotropics (primarily tricyclic antidepressants) were significantly more likely to be drug free at followup, independent of other variables. These results suggest that a subgroup of drug abusers benefit from appropriate use of psychotropic medications.

323.2 Effect of Cyclo(Leu-Gly) on Different Forms of Tolerance to Morphine. R.F. Ritzmann, J.M. Lee, and K.A. Steece. Alcohol and Drug Abuse Research and Training Center, University of Illinois Medical Center, Chicago, IL.

It has been proposed that different forms of tolerance to psychoactive drugs may develop. One scheme used to describe these forms of tolerance suggests 4 different types: Environmental Dependent Functional (EDF), Environmental Dependent Dispositional (EDD), Environmental Independent Functional (EIF) and Environmental Independent Dispositional (EID). The environmental dependent forms of tolerance are those forms which are associated with learning while environmental independent tolerance is the more traditional form of tolerance. The peptide cyclo(Leu-Gly) (cLG) has been shown to block functional tolerance to morphine. Due to the method used to induce tolerance (pellet implant) and the method of assessing tolerance (injection) this tolerance is considered EIF tolerance. On the other hand, data using a cue associated multiple injection paradigm to assess ED tolerance has indicated that cLG may alter this form of tolerance in a different manner than it alters EI tolerance. It does appear that the tolerance which develops to morphine using this method is primarily dispositional. Brain morphine levels in tolerance mice were 50% less than that which was found in non-tolerant animals. Therefore, it appears that it is EDD tolerance which is facilitated by cLG. Since cLG alters the various forms of tolerance which develop to this drug differently, it would appear that, at least as far as the peptides actions are involved, there are different underlying mechanism which mediate these processes. In addition, the different forms of tolerance which develop may account for the different results obtained in various laboratories which induce tolerance by different methods.

323.3 OPIOID EFFECTS ON TIMING BEHAVIOR IN THE RAT: POSSIBLE ACTIONS ON DOPAMINERGIC AND GABAERGIC NEURONS. Warren H. Meck* and Russell M. Church. W. S. Hunter Laboratory of Psychology, Brown University, Providence, Rhode Island 02912.

The purpose was to determine if the properties of an internal clock could be affected by the pharmacological manipulation of opiate and dopamine receptors. Emphasis was placed on the evaluation of morphine and its interaction with naloxone, methamphetamine, and haloperidol. A peak procedure was used to study the scaling of stimulus duration by rats (e.g., Meck & Church, J. Exp. Psychol. Anim. Behav. Processes 10: 1, 1984). In this procedure, after an intertrial interval a signal occurs and on food trials the rat's first lever press after a fixed duration is reinforced; on peak trials no food is available and the trial lasts for a relatively long time after the fixed duration. On peak trials the response rate of the rat initially increases as a function of the time since signal onset, as it does during standard fixed-interval training, and then it decreases in a fairly symmetrical fashion when plotted on a linear time scale. The time the response rate is maximal is called the "peak time." It occurs near the time that food is maximally expected by the rat. Using this procedure with reinforcement sometimes following the rat's first lever press after a white noise signal had been present for 20 s we demonstrated that morphine (0, 1, & 3 mg/kg) administered i.p. 20 min prior to an experimental session decreased the probability of attention to stimulus duration while at the same time it increased the sensitivity of the temporal discrimination on those trials that rats attended to time. These effects occurred in a dose dependent manner. No change in peak time was observed when morphine was administered alone, but morphine administration enhanced the temporary leftward shift in peak time produced by methamphetamine which has been interpreted as an increase in the speed of the internal clock due to an increase in the effective level of dopamine (e.g., Meck, J. Exp. Psychol. Anim. Behav. Processes 9: 171, 1983). The effects of morphine and morphine + methamphetamine were antagonized by naloxone. Morphine was without effect on the rightward shift in peak time produced by haloperidol blockade of dopamine receptors. Taken together, the data indicate a complex interaction between opiates, dopamine, and temporal processing. It is suggested that the effects of morphine on the speed of an internal clock are best explained by assuming that opiate administration inhibits GABAergic neurotransmission, thereby disinhibiting dopaminergic activity. (Supported by NIMH Grant MH 37049.)

323.4 ANTIHISTAMINES ALTER MORPHINE-INDUCED LOCOMOTOR HYPERACTIVITY. G. Andrew Mickley. Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814

Morphine causes a stereotypic locomotor hyperactivity in the C57BL/6J mouse. Although this behavioral response has been well described (Olivero, A. and Castellano, C., Psychopharmacol. 39:13, 1974), the neurochemical mediators which produce it have yet to be fully identified. Others have reported that brain histamine levels are inversely proportional to the morphine-induced locomotion of the mouse (Lee, J.R. and Fennessy, M.R., Clin. Exp. Pharmacol. Physiol. 3: 179, 1976; McClain, D.E., Catravas, G.N. and Teitelbaum, H., Soc. Neurosci. Abstr. 3: 297, 1977). Brain histamine may also be involved in various locomotor symptoms associated with opiate withdrawal (Wong, C. and Roberts, M.B., Agents and Actions 5/5: 476, 1975). The present investigation sought to further explore the role of brain histamine in opiate-induced locomotion by challenging this behavioral response with intracranial injections of antihistamines.

Eleven male C57BL/6J mice were chronically implanted with bilateral intracranial cannulas aimed at the lateral ventricles. After surgical recovery mice received morphine (30mg/kg, i.p.). On different days, amphetamine (4mg/kg, i.p.) or saline control injections were also given. The behavioral responses produced by the peripheral drug injection were, at different times, challenged by each of the following bilateral intraventricular injections: 1) 20mg chlorpheniramine (H_1 receptor blocker), 2) 75 mg cimetidine (H_2 receptor blocker, 3) 2 mg naloxone, or, 3) saline. Locomotor activity was recorded for 30 minutes after the intracranial drug injections.

Intraventricular naloxone significantly ($P < 0.05$, ANOVA) attenuated morphine-induced locomotion in this mouse. D-amphetamine hyperactivity was unaltered by the opiate antagonist. Chlorpheniramine also reduced morphine-induced locomotion (although not to the same degree as naloxone) but failed to produce a consistent reduction in amphetamine hyperactivity. Intracranial cimetidine evoked a generalized lethargy which reduced the behavioral activation produced by both morphine and d-amphetamine.

These preliminary data suggest a role for brain histamine in morphine stimulated locomotion. In addition, histamine's contribution to the production of morphine hyperactivity may differ from its role in amphetamine hyperactivity.

323.5 FEEDING ELICITED BY THE OPIATE PEPTIDE D-ALA-2-MET-ENKEPHALINAMIDE: SITES OF ACTION IN THE BRAIN. B. G. Stanley*, D. Lanthier* and S. F. Leibowitz (SPON: D. J. Micco). The Rockefeller Univ., 1230 York Ave., New York, NY 10021.

A possible role for brain opiate peptides in the regulation of feeding behavior was suggested by the findings that intracerebral microinjection of several opiates elicited feeding behavior. A fundamental question relating to this phenomenon concerns which brain region(s) is responsible for mediating these effects. To address this issue, the long acting enkephalin analogue D-Ala-2-Met-enkephalinamide (DALA), which has been shown to elicit feeding behavior (Mclean & Hoebel, 1983), was microinjected into various brain regions and its effect on food intake was measured.

Adult male Sprague-Dawley rats with stereotaxically-implanted 26 gauge cannulas were used. After satiation with fresh diet (46% Purina rat chow, 37% sucrose and 17% Carnation evaporated milk) subjects were injected through the brain cannula with DALA (4 µg/0.3 µl) or vehicle (0.9% saline, 0.3 µl) and food intake was measured 1 and 2 hr postinjection. Four to eight subjects per brain region were repeatedly tested with DALA and vehicle on alternating days. Subjects were considered to be responders if their mean food intake was increased 2 or more g over baseline.

As has been shown previously, we found that injection of DALA into the region of the paraventricular hypothalamus elicits a feeding response within 1 hr. This response was also observed with injection into the region of the perifornical hypothalamus and the amygdala. Other brain regions including the septum, globus pallidus, hippocampus, periaqueductal gray, midbrain tegmentum and fourth ventricle appeared to be insensitive to this opiate agonist.

These results suggest that opiate feeding is elicited through specific brain regions, in particular the hypothalamus and amygdala. In contrast, a number of other brain sites appear to be insensitive to this effect.

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323.6 DAY 10 RAT PUPS: EVIDENCE FOR ANALGESIC EFFECTS OF OPIOIDS. P. KEHOE and E.M. BLASS. Dept. of Psychology, The Johns Hopkins University, Baltimore, Md. 21218.

To determine the analgesic effects of opioids on neonatal rat nociceptive behavior, a hot-plate, paw-lift test was conducted on 10-day-old pups after various treatments. The first study examined the analgesic effect of morphine. Pups were given saline (s.c.) followed by morphine 0.5mg/kg (ip) 15 minutes later. Naltrexone 0.5mg/kg/saline, naltrexone/morphine and saline/saline were the other injection regimens Paw-lift latencies were tested at several intervals after the second injection. At the 15 minute interval we found increased latencies (27 sec.) after saline/morphine and considerably lower latencies for the other 3 treatment conditions. Apparently the opioid induced analgesia was blocked by naltrexone.

The second study demonstrated a stress-induced analgesia, presumably mediated by endogenous opioid release, which was antagonized by naltrexone. Prior to paw testing, each pup received either no injection, saline or naltrexone (.5mg/kg) and isolated in a cup of clean chips for 5 minutes. Latencies were 16, 18.5, and 7 sec. respectively. Similar results were obtained when the pup was isolated in orange-scented chips for 5 min. However, when group-housed in the orange-scented chips for 5 min., paw latencies were 9.5 sec. for no injection, 8.1 sec. for saline, and 5.6 sec. for the naltrexone group. Thus the analgesic effects of opioids are behaviorally functional in Day 10 rat pups exposed to a heated surface. This functional system, which was obscured in previous studies utilizing the tail-flick test was seen clearly in the present studies that used a test thought to be mediated, at least in part by brain opioid systems.

323.7 EFFECTS OF NALOXONE, MORPHINE AND DIAZEPAM ADMINISTERED PERIPHERALLY AND CENTRALLY ON PUNISHED RESPONDING. B.H. Herman+ and S.G. Holtzman*. Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322.

Rats were tested in the Geller-Seifter punishment task to determine if morphine has "anti-anxiety" effects and if naloxone has "anxiety-potentiating" effects. Central and peripheral routes of drug injection were compared. Diazepam was also studied. The test was a VI 1 min schedule of food reinforcement in which three 4-min punishment trials were presented alternately with four 12-min nonpunishment trials.

Regardless of the route of injection, morphine produced dose-dependent decreases in both operant components. For subcutaneously (s.c.) injected drug, the IC_{50} was 4.8 mg/kg (1,440 ug) for punished responding and 2.6 mg/kg (780 ug) for nonpunished responding. For intraventricularly (ICV) administered drug, the IC_{50}s were 27 nmol (7 ug) for punishment and 32 nmol (9 ug) for nonpunishment. Naloxone (1 mg/kg, s.c.) completely antagonized these effects.

No evidence for an "anxiety-potentiating" effect of naloxone was found. ICV injections induced nonselective rate-decreasing effects. The IC_{50} for tertiary naloxone on both operant components was greater than 300 nmol (98 ug). Quaternary naloxone was about 30 times more potent than the tertiary derivative (IC_{50} punishment = 11 nmol (3.0 ug), IC_{50} nonpunishment = 6.0 nmol (2.0 ug)).

ICV administered diazepam (1.0 and 35 nmol) or an s.c. injection (1.0 mg/kg) selectively increased the rate of punished responding without affecting nonpunished responding. These data suggest a central mode of action of diazepam.

In short, we failed to find a role of endorphins in the modulation of experimentally-induced "anxiety".

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323.8 The Effect of Morphine on Train Duration Thresholds and Response Rates in Self-Stimulating Rats. R. A. Frank and A. Markou. Dept. of Psychology, University of Cincinnati, Cincinnati, OH 45221.

Moderate doses of morphine (5-20 mg/kg) have been shown to initially depress self-stimulation followed by a facilitation of responding after 3-6 days of drug administration. The time course of the facilitory and depressive effects and their magnitude are dependent on both the time of testing and drug dose. Unlike most previous studies that have used either response rates or thresholds to assess the facilitory and depressive effects of morphine, the present study measured both rates and thresholds following drug administration. This approach was adopted to simultaneously determine the sensitivity of each measure to the bipolar effects of morphine. Ten male Sprague-Dawley rats implanted with bipolar stimulating electrodes in the ventral tegmental area (VTA) were trained to lever press for brain stimulation using a procedure that alternated 3.0 min test with 1.0 min time-out periods. The brain stimulation train duration that was available during each test period was varied from 15 to 70 msec in 5 msec increments. Train durations of 0 and 100 msec were also used. The 14 train durations were presented in a random order during each daily testing session. Two 1.0 hr testing sessions were run each day; the first 15 min and the second 3.0 hr post-injection. During pre- and post-drug baseline periods, normal saline was administered whereas either 5 or 10 mg/kg morphine was injected (SC) on each of 11 consecutive drug testing days. An examination of the data revealed that, in general, increased rates were associated with decreased thresholds and decreased rates with increased thresholds. The results of this experiment indicated that (1) morphine affects self-stimulation response rates and thresholds in approximately the same manner, (2) a simple motor explanation of morphine's depressive or facilitative effects on self-stimulation is inadequate and (3) individual differences in response to morphine can be substantial and must be considered in any general explanation of morphine's effect on self-stimulation. This research was supported by a University of Cincinnati URC grant to R. Frank.

323.9 FACILITATION OF SELF-STIMULATION BY MORPHINE: AN ASSOCIATIVE RATHER THAN A PHARMACOLOGICAL PROCESS? T.H.Hand* and K.B.J. Franklin. Department of Psychology, McGill University, Montreal, Quebec H3A 1B1

In recent years it has become apparent that a number of the physiological and behavioral effects of chronic morphine are controlled by environmental stimuli associated with drug administration. The facilitation of self-stimulation (SS) by daily morphine injections develops over a period of several days, suggesting that it is not a simple, direct pharmacological effect of the drug. The hypothesis that this effect represents the development of a learned association between morphine administration and the SS situation was tested in 2 experiments. In Experiment 1, rats were trained to self-stimulate, and then stabilized at currents producing half-maximal rate for several consecutive days. The animals were then randomly divided into 2 groups. Group A received 10 mg/kg morphine SO_4 subcutaneously each day, and SS performance was tested 1h and 3h post-injection. This procedure was followed for 8 days. Group B received the same dose of drug in the animal colony for 4 days, and were not returned to the SS procedure until the 5th day of morphine injection. Group A showed a gradual development of SS facilitation at 3h post-injection. This was detectable as early as the second day of morphine administration and very strong by days 3 and 4. Group B showed no facilitation, despite repeated testing. This suggested that facilitation of SS by morphine requires repeated pairings of the drug and the SS situation, and that removal of the association between the drug and the SS situation blocks this effect. In Experiment 2, a group of animals was trained and tested under the same conditions as Group A above, except that the drug-SS association was weakened by testing only 3h post-injection (rather than 1h and 3h post-injection). Under these conditions, chronic morphine failed to affect SS performance over 5 days of drug treatment. Introduction of the 1h post-injection session on days 6-10 of SS did not initiate facilitation. These data show that repeated opiate receptor activation by morphine is not a sufficient condition for SS facilitation, and they support the notion that this facilitation is under the control of environmental stimuli associated with drug administration.

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323.10 EFFECTS OF 0.5 HZ AND 60 HZ MAGNETIC FIELDS ON MORPHINE-INDUCED BEHAVIORAL CHANGES IN MICE. M. Kavaliers and K.-P. Ossenkopp (SPON: R. Shivers). Depts. of Zoology and Psychology, University of Western Ontario, London, Ontario, Canada N6A 5B7

A substantial body of evidence indicates that magnetic fields can influence biological systems. Of special interest is the extremely low frequency band in the range of 50-60 Hz since this is the frequency of alternating currents used as sources of electrical energy by man. Although there is some evidence for increased activity levels in mice exposed to magnetic fields with a 60 Hz frequency, few other reliable behavioral changes have been reported. Recently, chronic exposure to 0.5 Hz magnetic fields was shown to markedly attenuate nocturnal morphine-induced analgesia in mice. We report here that acute (1 h) exposure to 0.5 Hz and 60 Hz magnetic fields also reduced murine nocturnal and diurnal morphine-induced analgesia as well as reducing morphine induced hyperactivity.

CF-1 mice given morphine display day-night rhythms in the latency of their response to a thermal stimulus (50°C). In experiment 1, 1 h exposure to 0, 0.5, 1.0 and 1.5 gauss 60 Hz magnetic fields reduced in a dose-dependent fashion the normally elevated nocturnal analgesia levels in CF-1 mice that were i.p. treated with morphine sulfate (10 mg/Kg). In experiment 2, exposure of CF-1 mice for 1 h to a 0.5 Hz magnetic field (3-90 gauss) was found to also reduce the day-time morphine (10 mg/Kg)-induced analgesic responses. In the final experiment CJ-57 mice were exposed to the 0.5 Hz magnetic field conditions and their activity levels were measured after treatment with morphine (10 mg/Kg). In contrast to the CF-1 mice the CJ-57 strain of mouse displays hyperactivity rather than analgesia after morphine treatment. A significant reduction in morphine-induced activity was observed in the CJ-57 mice exposed to the magnetic fields.

These experiments show that exposure to magnetic fields can signficantly alter morphine-induced behavior in mice. These results are compatible with a magnetic-field-induced inhibition of pineal gland activity hypothesis.

323.11 **EFFECTS OF 5,7-DIHYDROXYTRYPTAMINE LESIONS OF THE NUCLEUS ACCUMBENS ON RAT INTRAVENOUS MORPHINE SELF-ADMINISTRATION.** **J.E. SMITH and G.F. GUERIN*. Psychiatry Research Unit, Departments of Psychiatry and Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.**

The nucleus accumbens (NA) is thought to be an important brain structure to stimulant and opiate reinforcement. Bilateral 6-OHDA and kainic acid lesions decrease intravenous cocaine and amphetamine self-administration, but either do not effect or increase opiate intake. Bilateral 6-OHDA lesions also attenuate opiate and amphetamine place-preference conditioning without effecting cocaine, suggesting a heterogenity of function. 5,7-dihydroxytryptamine (5,7-DHT) lesions of the NA do not change intravenous amphetamine self-administration. This experiment was initiated to assess the effects of similar serotonergic lesions on intravenous morphine self-administration. Six pairs of 90-day-old male Fischer F-344 littermates were surgically implanted with intravenous jugular catheters and bilateral injection guide cannulae into the central medial nucleus accumbens, were made physically dependent on morphine and then allowed to lever press on a FR-10 schedule of intravenous drug presentation (10 mg/kg in 0.2 ml delivered over 5.5 sec with 24-hour access). After stable baselines of drug intake were obtained (2-3 weeks) one of each pair received bilateral sham vehicle treatment while the other received bilateral 5,7-DHT microinjections into the nucleus accumbens (6 ug in 0.5 ul of saline with 0.2 mg/ml of ascorbic acid infused over 7-1/2 minutes with the injection cannulae left in place an additional 10 minutes) 30 to 45 minutes after desmethylimipramine pretreatment (25 mg/kg, i.p.). The littermates received response independent infusions of morphine for 24 hours at the previous self-administration rate and then again allowed to self-administer with drugintake monitored for thirteen days. The littermates were sacrificed by total immersion in liquid nitrogen, the brains removed at -20°C, frozen sections of the cannulae tract taken for histological assessment, the nucleus accumbens removed at -20°C and biogenic monoamine content determined by HPLC with electrochemical detection. 5,7-DHT lesions resulted in a significant increase in drug intake (75%), while the sham vehicle treatment did not. Content of 5-HT and 5-HIAA was also significantly reduced in the lesioned animals. The increase in drug intake is similar to that seen after 6-OHDA lesions, suggesting a role for 5-HT innervations of the NA in opiate reinforcement. (Supported by USPHS Grant DA-01999).

323.12 OPIOIDS AND AGGRESSION: INFLUENCES OF OPIOID AGONISTS AND ANTAGONISTS ON AGGRESSIVE BEHAVIOR IN MICE. G.C. Teskey and M. Kavaliers. Dept. of Zoology, University of Western Ontario, London, Ontario, Canada N6A 5B7.

A growing body of evidence has suggested a role for endogenous opioid peptides in the control of aggressive behavior. Aggressive interactions follow a defined temporal course and are composed of a repertoire of behavioral elements. The present study was undertaken to examine the roles of different components of the opioid system in mediating these behaviors. Determinations were made of the effects of various opioid agonists and antagonists on the composition and behavioral consequences of aggressive encounters in mice.

The effect of size, territory, and previous social experience on aggressive behavior was investigated using "resident-intruder" encounters. Opioid agonists for mu, delta, and kappa receptors as well as the opioid antagonist, naloxone, were administered to either the resident or intruder mouse. Behavioral interactions of various combinations of mice from two size classes (20-25 g and 35-40 g), on three territories (home, neutral, and foreign), and from two different housing conditions (grouped and isolated) were examined. Aggressive interactions were observed over a 10 minute period and a number of behavioral components were recorded. These include, latency to attack, number of bites, number of bites to produce defeat, frequency of attack, and number of bouts. In addition, aversive thermal resopnse latencies, locomotor activity, and feeding behaviors were measured following the aggressive interaction. This permitted a further evaluation of the relative involvements and activation of different opioid receptor arising from during the aggressive encounters.

The temporal sequence and aggressive components were consistent within a paradigm but differed greatly between paradigms. In parallel there were also variations in relative opioid involvement in the determination of the subsequent feeding, activity, and aversive responses.

323.13 EFFECTS OF PENTOBARBITAL ON THREE FORMS OF STRESS-INDUCED ANALGESIA. M.V. Klein*, G.W. Terman and J.C. Liebeskind. Department of Psychology, UCLA, Los Angeles, CA 90024.

A variety of stressors has been implicated as stimuli activating endogenous pain-inhibitory systems within the brain and spinal cord. We have recently found that by varying the parameters of an inescapable footshock stressor at least three distinct forms of stress-induced analgesia can be differentially produced. Whereas, 1 min of continuous or 20 min of intermittent (1 sec on every 5 sec) 2.5 mA footshock elicits an analgesia that by several criteria appears to be mediated by opioid peptides, 4 min of continuous footshock at the same intensity produces an equipotent analgesia independent of opioids. The two opioid paradigms of stress analgesia differ from one another, in that the 20 min paradigm produces analgesia attenuated by hypophysectomy and adrenalectomy, whereas the analgesia from 1 min of footshock is independent of these manipulations. Further, although all three forms of stress analgesia depend on supra-spinal structures, in that they are blocked by spinal transection, only the 20 min intermittent stress analgesia depends on supra-mesencephalic structures and can be attenuated by mid-collicular decerebration. In this study, the effects of sodium pentobarbital on these three forms of stress analgesia were examined.

Male Sprague-Dawley rats were administered, 0, 10, 20, 30, 40, 50, or 60 mg/kg of sodium pentobarbital (i.p.) (n=6). Animals were tested for baseline nociceptive responsiveness using the tail-flick test and footshocked for 1 or 4 min continuously or 20 min intermittently. Footshock for all animals ended 40 min post-injection. Post-stress tail-flick tests were conducted at 1 min intervals for 10 min.

Pentobarbital was found to significantly attenuate 20 min stress analgesia at doses of 40, 50 and 60 mg/kg when compared to saline controls, whereas 10, 20 and 30 mg/kg had no effect on this analgesia. Animals shocked with either 1 or 4 min of continuous footshock showed no significant analgetic differences from saline injected controls, after 10, 20, 50, or 60 mg/kg. However, animals injected with either 30 or 40 mg/kg doses demonstrated significantly less stress analgesia. (Supported by NIH grant NS07628 and a gift from the Brotman Foundation).

323.14 IMPORTANCE OF STRESS SEVERITY IN DETERMINING THE OPIOID OR NONOPIOID NATURE OF COLD SWIM-INDUCED ANALGESIA. G.W. Terman, M.J. Morgan* and J.C. Liebeskind. Department of Psychology, UCLA, Los Angeles, CA 90024.

Much evidence supports the hypothesis that systems exist within the brain and spinal cord whose normal function is the inhibition of pain. Stress has been suggested to be a natural trigger for such endogenous pain-inhibitory systems. We have recently found that exposure to one stressor, continuous inescapable footshock, can elicit analgesia in rats or mice which, as a function of the temporal and intensive parameters, depends either on opioid peptides or is independent of these neurochemicals. Whereas shorter or weaker footshock caused opioid analgesia, longer or stronger footshock caused nonopioid analgesia. The present experiment was undertaken to examine whether stress severity might also determine the opioid or nonopioid nature of analgesia elicited by another stressor, cold water swim.

Male Sprague-Dawley rats were divided into 4 groups (n=12) and, after baseline pain testing (using the tail-flick test), were placed in 40° C water for 5 min, 15° C water for 5 min or 10 min, or 10° C water for 5 min. Twenty min prior to water exposure, half of each group was injected with naltrexone (5 mg/kg, s.c.). Following removal from the water, core temperatures were taken and all animals received 10 tail-flick trials at 1 min intervals.

Whereas no significant analgesia was seen following exposure to 40° C water for 5 min, all other swim conditions did produce analgesia. Naltrexone significantly attenuated only the analgesia following exposure to 15° C water for 5 min. Increasing either the intensity (to 10 C) or duration (to 10 min) of cold water exposure, resulted in an analgesia unaffected by naltrexone. Swim conditions yielding naltrexone-insensitive analgesia produced lower core temperatures. Naltrexone had no effect on post-swim temperatures for any swim condition.

Thus, increasing the severity of cold water exposure, like increasing footshock severity, changes the neurochemical basis of the resulting stress analgesia from opioid to nonopioid. (Supported by NIH grant NS07628 and a gift from the Brotman Foundation).

323.15 LACK OF CROSS-TOLERANCE TO SYSTEMIC MORPHINE INDUCED ANALGESIA AFTER CHRONIC INTRATHECAL INFUSION IN RATS. C.B. Tyler and C.D. Advokat. Dept. Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612

Constant spinal infusion of opiates has proven to be a valuable treatment for chronic (usually cancer) pain. Surprisingly, several reports have shown that in contrast to the systemic route of administration, minimal tolerance develops to the analgesic effect with constant infusion over days and sometimes months. In the process of examining the question of tolerance to spinal vs systemic opiates, we have previously reported (Neurosci. Abst. 8: 355: 1982; Comm. on Problems of Drug Dependency, June, 1984) that the analgesic effect of intrathecal (i.th.) morphine in animals made tolerant to systemic morphine is influenced by the nociceptive assessment test. Rats that are tolerant to systemic morphine on the hot plate (HP) are also tolerant to i.th. morphine; while rats that are tolerant to systemic morphine on the tail flick (TF) are not tolerant to i.th. morphine. In order to further explore the interaction between the development of tolerance and "cross-tolerance" to opiates, the route of administration and the nociceptive test employed, we examined the development of "cross-tolerance" to systemic morphine following chronic i.th. morphine infusion.

Male albino rats (350-400 gm) were implanted with spinal catheters. After recovery, animals received 3 non-drug tests on either the HP or the TF. Animals were then implanted subcutaneously (sc) with an osmotic mini pump (Alzet Model 2001; flow rate 1 µl/hr for 1 week) which was connected to the spinal catheter. The pumps were filled with either saline, 25, 50 or 70 µg/µl. Rats were tested daily until the latencies returned to baseline. "Cross-tolerance" was assessed by an acute sc injection of either 3 mg/kg (TF) or 6 mg/kg (HP) morphine followed by a final test 40 minutes later. The results indicated: 1) the 70 µg/µl dose was toxic and the data from these rats was not included in the analysis and 2) that the two morphine groups did not differ from the saline control group on either test. Therefore, no "cross-tolerance" was obtained to systemic morphine after chronic spinal infusion.

In concert with previous results these data suggest that the level of drug action (spinal vs supra-spinal) and the neural substrates mediating the nociceptive behavior interact to affect the development of "cross-tolerance". We are currently investigating whether a similar effect on tolerance is obtained to an acute i.th. injection after chronic spinal infusion of morphine. (Supported by PHS Grants DA 38053 and DA 02845)

323.16 STUDIES ON IPSILATERAL CIRCLING IN RATS INDUCED BY UNILATERAL INJECTIONS OF OPIATES INTO THE VENTRAL TEGMENTAL AREA. M.R. Szewczak* and M.T. Spoerlein. Rutgers University, P.O. Box 789, Piscataway, N.J. 08854.

Previously, we reported that morphine and ethylketocyclazocine cause dose-dependent ipsilateral circling behavior in rats when injected unilaterally into the ventral tegmental area (VTA); this circling was not sensitive to a relatively high dose of naloxone (10mg/Kg, ip). (Szewczak and Spoerlein, Soc. of Neurosci. Abstr. 9:279, 1983.)

We are now reporting that the circling caused by these compounds is blocked by the narcotic antagonist, diprenorphine (10 mg/Kg, ip), suggesting that this behavior might be mediated by a non-mu opiate receptor. Additionally, in rats made tolerant to morphine (2-50 mg pellets for 72 hrs.), circling caused by ethylketocyclazocine but not morphine was diminished in animals with pellets removed and undergoing withdrawal. In animals with pellets left intact, the circling behavior seen with both morphine and ethylketocyclazocine was unchanged. These results again suggest interactions at a non-mu opiate receptor located in the VTA.

(Supported in part by a Charles and Johanna Busch Research Grant - Rutgers University.)

323.17 THE EFFECTS OF HEROIN ON BRAIN-STIMULATION REWARD. C.B. Hubner and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118

Increased sensitivity for rewarding brain stimulation has been used as an animal model of drug-induced euphoria and is thought to be predictive of abuse liability in man. Abused substances including morphine, amphetamine and cocaine have been shown to lower brain-stimulation reward thresholds (Kornetsky and Esposito, Fed Proc., 38: 2473-2476, 1979). The present study was conducted to determine if heroin, a drug with high abuse potential and which has been reported to increase lever pressing for intracranial stimulation (Koob et al., Psychopharmacology., 42:231-234, 1975), would also significantly lower the reward threshold.

Male albino rats (CDF-Charles River Laboratories) were stereotaxically implanted with bipolar stainless steel electrodes aimed at the medial forebrain bundle-lateral hypothalamic area. Determination of the brain-stimulation reward thresholds was accomplished by using a variation of the psychophysical method of limits. Heroin (0.03-0.5 mg/kg sc) produced a dose-dependent lowering of the reward threshold for intracranial self-stimulation.

It has been reported that heroin is between 2-10 times a more potent analgesic than morphine in animals and man (Umans and Inturrisi, J. Pharmacol. Exp. Ther., 218:409-415, 1981; Reichle et al., J. Pharmacol. Exp. Ther., 136:43-46, 1962). In our laboratory we have found that the minimally effective dose of morphine that will lower the reward threshold is 2-4 mg/kg, while in the present study we found that the minimally effective dose of heroin that lowers the threshold is 0.06 mg/kg. These results indicate that heroin is approximately 30 times more potent than morphine in causing increased sensitivity to rewarding brain stimulation. If a lowering of the threshold for intracranial stimulation is an indication of drug-induced euphoria, these results would suggest that given equal potent analgesic doses, heroin will cause greater euphoria than morphine.
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323.18 ACTIVE INVOLVEMENT OF ENDORPHINS IN THE PRODUCTION OF TOLERANCE TO ANALGESIA INDUCED BY INTERMITTENT COLD WATER STRESS IN RATS. F.A. Holloway & M.N. Girardot*. Department Psych. & Behav. Sci., Oklahoma Univ. HSC, Oklahoma City, OK

We have shown that intermittent cold water stress (ICWS, 18 exposures, 10 sec each, 3/min) induces an analgesia which is cross-tolerant to morphine, suggesting that ICWS-analgesia is an opiated-mediated phenomenon. However, our finding that rats tolerant to morphine are not tolerant to ICWS ruled out the hypothesis that the same mechanisms mediate morphine and ICWS-tolerance. This study was aimed toward further analysis of mechanisms involved in ICWS-tolerance. Male Sprague-Dawley rats were submitted to ICWS on 16 consecutive days. On Days 15 and 16, they were injected i.p. with saline and naltrexone (10 mg/kg), respectively. Analgesia was measured using the tail-flick test prior to and 30 min after daily ICWS. Tolerance to ICWS-analgesia developed, and was significantly reversed by naltrexone. The tolerance-reversible effect of naltrexone generalized to the other effects of ICWS to which adaptation also developed: core hypothermia, hypoactivity, and a type of behavior which appeared on Day 4-5 of ICWS (passivity, horizontal floating). Naltrexone had no effect on those variables studied for which no adaptation was found (skin hypothermia, intensively active escape behavior, passivity, vertical floating). This suggested that tolerance to ICWS may result from an active involvement of endorphins. Such a paradoxical role of endorphins in chronic stress was confirmed by effects of morphine in rats submitted to ICWS on 14 consecutive days. These rats displayed tolerance to ICWS and a biphasic response on the tail-flick test after 10 mg/kg morphine, i.e., a short-latency hyperalgesia was followed by a longer-latency analgesia which was significantly weaker than in non-stressed rats. The short-term hyperalgesic effect of morphine was found with doses ranging from 2.5 to 10 mg/kg. The long-term analgesic effect of morphine was reduced. These results suggest that a type of opiate receptor is progressively sensitized by chronic ICWS, which results in: i) the production of ICWS-tolerance through stress-released endorphins, and ii) a hyperalgesic effect and reduced ability of exogenously administered opiates to induce analgesia. These findings provide empirical validation for the opponent-process theory of tolerance (Solomon, 1980) according to which, during chronic exposure to a drug or stressor, tolerance results from the development of a process which actively opposes acute effects of the treatment.

323.19 DIFFERENTIAL PATTERNS OF ANALGESIA PRODUCED BY MORPHINE AND KETOCYCLAZOCINE IN THE DEVELOPING RAT. J. Giordano*, G.A. Barr, M-C. Poupard* & R.S. Zukin (Spon: J. Nazzaro). Albert Einstein College of Medicine and Biopsychology Program, Hunter College, CUNY, New York, N.Y. 10021.

Our laboratory has demonstrated that kappa opiate receptor mediated analgesia is detectable in the young rat four days earlier than is mu opiate mediated analgesia in the tail flick test using thermal noxious stimuli. Neural circuits for nociception have been shown to differ for forepaw, hindpaw, and tailflick responses; further kappa opiate receptors may be more selective than mu receptors in blocking mechanical nociceptive signals. The present study examined the ontogenetic pattern for the analgesic response produced by the prototypic mu agonist morphine (M) and the prototypic kappa agonist ketocyclazocine (KC) using both thermal and mechanical noxious stimuli in these three paradigms.

Drugs were administered intraperitoneally to 3,10,14, or 21 day old rat pups and response latencies to a thermal (hot water, 50° C) or mechanical noxious stimulus (dulled 23 ga. needle) were measured. In the case of a thermally noxious stimulus applied to the forepaws, an analgesic response to morphine was evident at 3 days of age. In the case of thermal stimulation to the tail, morphine first produced analgesia at 14 days of age; morphine did not produce significant analgesia to thermal stimulation of the hindpaws at any age. In contrast, in the case of a mechanical stimulus, adult levels of analgesia appeared first at 10 days of age for each body part.

KC also produced adult levels of analgesia when the thermal stimulus was applied to the forepaw of 3 day olds, but again failed to produce analgesia when thermal stimuli were applied to the hindpaw. The ability of KC to block the tail flick response, however, appeared at adult levels by 10 days of age, regardless of the type of stimulus.

The ontogenetic pattern of opiate-induced analgesia was correlated with the development of mu and kappa opiate receptors. The present studies serve to define further the functional differences between mu and kappa receptors in the mediation of drug induced analgesia.

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323.20 EFFECT OF PG SYNTHETASE INHIBITORS ON MORPHINE-INDUCED EXCITATION OF CNS IN RAT. M.C. Wallenstein. Dept. Physiology, New York University, NY NY 10010.

Prostaglandin (PG) synthetase inhibitors attenuate morphine-induced catalepsy, exophthalmos, mydriasis, and inhibit morphine-induced hyperthermia in rat (Wallenstein, 1983). Morphine also induces dose-related excitation in the rat leading to seizure. To investigate whether PGs play a role in this action of morphine, the electrocortical activity and behavior of rats with chronically-implanted electrodes were studied. A 60 mg/kg dose of morphine ip produced electrocortical bursts of high voltage activity during which the animals showed no spontaneous body movements. Two out of 5 animals showed isolated myoclonus. A dose of 250 mg/kg morphine ip produced electrocortical spikes and myoclonus leading to seizures. Pretreatment with paracetamol (450 mg/kg) or ibuprofen (30 or 90 mg/kg) significantly delayed and/or blocked morphine-induced seizures. In contrast, mefenamic acid or meclofenamic acid (15 or 50 mg/kg) significantly increased the amount of myoclonus induced by 60 mg/kg morphine and decreased the onset latency to electrocortical seizures induced by 250 mg/kg morphine. This differential effect of individual PG synthetase inhibitors may reflect: 1) PG synthetase systems with different pharmacological characteristics; 2) a variety of actions by the PG synthetase inhibitors in addition to blocking PG synthesis.

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OPIATES, ENDORPHINS, AND ENKEPHALINS: PHYSIOLOGICAL EFFECTS II

324.1 NICOTINIC REGULATION OF THE SECRETION OF IMMUNOREACTIVE MET-ENKEPHALIN. G.R. Van Loon and S. Mousa*. VA Medical Center and Department of Medicine, University of Kentucky, Lexington KY 40511.

Met-enkephalin and a number of proenkephalin A-derived peptides are localized in chromaffin cells and splanchnic nerve terminals of the adrenal medulla. In addition, Met-enkephalin is distributed in sympathetic ganglia and a number of pre- and postganglionic sympathetic nerves. Co-secretion of enkephalin-related peptides and catecholamines has been demonstrated from chromaffin cells in culture and from isolated perfused adrenal glands in response to either nicotine or acetylcholine. In this study, we compared the effects of acute and chronic administration of nicotine to adult male Fisher rats on the in vivo secretion of Met-enkephalin.

Nicotine, 1 mg/kg, was administered acutely intraarterially in chronically cannulated rats, and blood collected at intervals from the cannula. Also, nicotine, 1 mg/kg, was administered acutely subcutaneously in noncannulated rats, and trunk blood collected at decapitation. Nicotine was administered chronically for one month in drinking water, and food and water intake and body weight were monitored; daily nicotine intake approximated 0.9 mg per rat. In this study, trunk blood was collected at decapitation. Adrenal glands were dissected and homogenized in 1N HCl containing 1% disodium ethylenediamine tetraacetate. Plasma and adrenal concentrations of Met-enkephalin-like immunoreactivity (irME) were assayed by radioimmunoassay using a rather specific antibody which does not cross-react with other enkephalin-related peptides. Plasma epinephrine and norepinephrine were assayed radioenzymatically, and adrenal epinephrine and norepinephrine were assayed by high pressure liquid chromatography with electrochemical detection.

The acute systemic administration of nicotine increased plasma concentrations of irME, epinephrine and norepinephrine without affecting the adrenal concentrations. The plasma irME response to intraarterial nicotine was greater than that to subcutaneous nicotine and peaked within 2 minutes after administration. In contrast, the chronic oral administration of nicotine resulted in decreased plasma concentration of irME. Thus, the secretion of irME produced by initial exposure to nicotine is altered during chronic exposure to nicotine.

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324.2 EFFECT OF MU- AND DELTA-OPIOID RECEPTOR AGONISTS ON CARDIORESPIRATORY AND METABOLIC FUNCTION. J.I. Schaeffer* and G.G. Haddad.* (Spon: Salvatore DiMauro) Dept. of Pediatrics. Columbia University-College of P&S, New York, NY 10032.

To investigate the role of endogenous opioids in regulating cardiorespiratory function, we studied ventilation, ventilatory pattern, heart rate, arterial blood pressure, O_2 consumption (VO_2) and arterial blood gas tensions in chronically instrumented, unanesthetized dogs after intracisternal administration of opioids. Morphiceptin analog (MA)(Tyr-Pro-NMePhe-D-Pro-NH_2), a very selective mu-receptor opioid agonist, and D-Ala-D-Leu-Enkephalin (DADLE), a preferential delta-receptor opioid agonist were administered in doses of 5,25 and 125 mcg/kg. We performed 28 studies in 9 adult dogs. Ventilation was measured by barometric plethysmography and VO2 by a closed circuit system.

DADLE increased expiratory time (Te) and decreased instantaneous minute ventilation (Vt/Ttot) for about 2 hours; the effect was dose-dependent. In contrast, MA decreased Te and tidal volume with a resultant increase in Vt/Ttot. Both MA and DADLE increased the number of sighs/unit time. The RR interval (inverse of heart rate), after an initial transient drop, increased and reached a peak at about 25-60 minutes after both MA and DADLE. However, DADLE produced a more prolonged and marked change in RR interval than MA. After both agonists, mean arterial blood pressure (MAP) increased during the first 10 minutes by 10-40% but returned to baseline by 20-30 minutes, a time when the RR interval was still increasing. Both the mu- and delta-agonists produced profound decreases in VO_2 (20-50%). Arterial pCO_2 increased and pO_2 decreased modestly after both agonists. Intracisternal naloxone (dose: 0.01-20.0 mcg/kg) reversed the cardiovascular and respiratory effects when injected post agonist but had no effect when injected alone.

These data suggest that: 1) although the effect of the mu-opioid receptor agonist on cardiovascular and metabolic function is similar in direction to that of the delta-opioid receptor agonist, they have opposite effects on total ventilation; 2) both agonists induce alveolar hypoventilation. This results from the decrease in total ventilation after delta-opioid agonist and the increase in dead space ventilation after mu-opioid agonist and 3) endorphins do not tonically modulate ventilatory, cardiovascular and metabolic function.

324.3 MICTURITION IN CONSCIOUS DOGS FOLLOWING SPINAL OPIATE ADMINISTRATION. J M Bolam*, C J Robinson, and R D Wurster (Spon: T Khan). Hines VA Rehab R&D Center, Hines,IL 60141 & Dept of Physiology, Loyola Univ Sch of Medicine, Maywood,IL 60153.

Micturition dysfunction is a major side effect when opiates are used spinally for pain relief. The presence of opiate receptors in the lateral sacral spinal cord (Glazer and Bausbaum,JCN '81) may be one basis for this dysfunction. We tested this hypothesis by performing repeated slow-fill (16 ml/min) retrograde water cystometry on six dogs weighing 20 to 24 kg. We compared the effects of 1 mg intrathecal (IT) morphine sulfate (with and without preservative) and 0.4 mg naloxone HCl, with those effects produced by IV injections of 1 and 10 mg morphine and 0.4 mg naloxone. Each dog was tested about once a week for 5 to 8 weeks, with a different intervention each week. Before each session, we installed an IT catheter through a lumbar puncture and catheterized the bladder, all under halothane anesthesia (2% in 100% 02). A control cystometrogram was performed one hour or more after anesthesia was discontinued, with the animal awake in a familiar restraining sling. Additional cystometrograms were performed after drug administration. Reflex responses to pin prick were noted for all limbs after each cystometry.

IT morphine significantly ($p<0.05$) increased the micturition volume threshold. IT naloxone, alone or following IT morphine injection, significantly reduced this threshold, in most cases to below control volumes. IV morphine raised thresholds in a dose dependent manner, but reversal by IV naloxone was not as marked as with IT naloxone. No significant difference existed between control and post-drug treatments regarding the average quiescent pressure or volume-pressure slope of the cystometrogram, or peak micturition pressure. However, moment to moment pressure fluctuations (reflecting detrussor stability) were generally reduced after IT morphine and increased after IT naloxone. By novel use of Rasch psychometric analysis (Wright & Masters, MESA Press, Chicago, '81), we could analyze reflex changes even though not all animals received all treatments. IT morphine reduced hindlimb pin prick reflexes, while a similar amount given IV did not. IT naloxone, when given to reverse a morphine injection, produced hyperreflexic responses to hindlimb pin prick, while IT naloxone given without a previous morphine injection had no effect on reflex behavior.

Our results suggest that the bladder dysfunction seen following spinal opiate administration is of spinal origin.

(Supported by the Veterans Administration).

324.4 CONTRASTING EFFECTS OF OPIATE AGONISTS AND AGONIST-ANTAGONISTS ON ADENYLATE CYCLASE AND EXCITABILITY IN SYMPATHETIC PREGANGLIONIC NEURONS. Parley W. Madsen, Bradford D. Hare*, and Donald N. Franz. Departments of Pharmacology and Anesthesiology, University of Utah, Salt Lake City, Utah 84132.

Our previous studies have shown that clonidine and opiate agonists depress sympathetic preganglionic neurons (SPGNs) by activating alpha-2 and opiate receptors, both of which are negatively coupled to adenylate cyclase (Science 215:1643, 1982; Soc. Neurosci. Abstr. 9:797, 1983). The present study compared the effects of two opiate agonist-antagonists, pentazocine and butorphanol with those of two opiate agonists, morphine and methadone, on SPGN excitability and adenylate cyclase activity. Sympathetic discharges recorded from upper thoracic preganglionic rami were evoked at 0.1 Hz by stimulating descending excitatory pathways in the cervical dorsolateral funiculus of unanesthetized, spinal cats.

Inhibition of cyclic AMP phosphodiesterase (PDE) by aminophylline (50 mg/kg), IBMX (1 mg/kg), or RO 20-1724 (1 mg/kg) enhanced intraspinal transmission to 175% of control values within 10 min. Pretreatment with morphine (2 mg/kg) or methadone (1 mg/kg) rapidly depressed transmission to 0-40% of control and almost completely prevented enhancement by the PDE inhibitors. The opiate antagonists, naloxone or naltrexone (20-40 ug/kg), rapidly reversed the depression by the agonists and restored the ability of the PDE inhibitors to enhance transmission. In contrast, pentazocine (2 mg/kg) or butorphanol (1 mg/kg) produced only a mild depression (10-15%) of transmission to SPGNs that was not reversed by the antagonists. Furthermore, these drugs did not alter the rapid enhancement of transmission produced by the PDE inhibitors. The ability of opiate agonists, but not agonist-antagonists to depress SPGN excitability and to inhibit adenylate cyclase indicates that the opiate receptors on SPGNs that are negatively coupled to adenylate cyclase are mu-receptors rather than kappa- or sigma-receptors. Opiate agonists may lower blood pressure by depressing SPGNs.

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324.5 DYNORPHIN A REDUCES VOLTAGE-DEPENDENT CALCIUM-CONDUCTANCE OF SENSORY NEURONS: A VOLTAGE CLAMP STUDY. R.L. Macdonald and M.A. Werz. Dept. of Neurology, University of Michigan, Ann Arbor, MI 48104.

We previously reported that dynorphin A (DYN) decreased somatic calcium-dependent action potential (CAP) duration in a portion of mouse dorsal root ganglion (DRG) neurons. DYN action was antagonized by naloxone. Responses of DRG neurons to DYN, unlike responses to Leu-enkephalin (L-ENK), a δ- preferring ligand, or morphiceptin, a μ-selective ligand, were associated with decreased CAP after-hyperpolarization and persisted following intracellular injection of cesium, a potassium channel blocker. Therefore, we suggested that μ- and δ-opioid receptors are coupled to voltage- and/or calcium-dependent potassium channels but that DYN acts at a distinct opioid receptor, possibly the κ-opioid receptor, that is coupled to voltage-dependent calcium channels. In the present investigation we directly assessed DYN actions on calcium and potassium currents using the single electrode voltage clamp technique.

Cell culture and opioid application techniques were as previously described (J.Pharmac.Expt.Ther., 227:374, 1983). The somata of DRG neurons were voltage clamped using a single microelectrode voltage clamp preamplifier (Axoclamp 2).

Step depolarizations of neurons bathed in medium substituting the potassium channel blocker cesium for potassium and impaled with micropipettes filled with 3M CsCl, evoked calcium-dependent currents that only partly decayed over a 75 msec period. DYN at 1 μM decreased calcium inward currents in 13 of 38 neurons. In contrast, L-ENK at 5 μM did not affect calcium currents in 11 of 11 neurons including 5 neurons that responded to DYN. DYN decreases of calcium currents were antagonized by 1 μM naloxone (n=5). DYN did not affect threshold potential for the onset of the inward current, the potential at which the inward current was maximal, or the reversal potential for calcium current (n=5). When the inward calcium current was blocked by cadmium, the remaining leak current was unaffected by DYN. The decreases in inward current by DYN at step commands that fully activated calcium conductance were associated with a reduction of chord conductance. We suggest that DYN (κ)-receptors are coupled to voltage-dependent calcium channels. However, μ- and δ-receptors are likely to mediate actions via enhancement of potassium conductance.

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324.6 ACTION OF OPIOID PEPTIDES ON FAST-PHASE CALCIUM UPTAKE INTO BRAIN REGIONAL SYNAPTOSOMES. E. Barr and S.W. Leslie. Division of Pharmacology, College of Pharmacy, University of Texas, Austin, TX 78712

Uptake of calcium by synaptosomes isolated from hippocampus (HIP), frontal cortex (FC) and striatum (STR) of male Sprague-Dawley rats was measured at three seconds. Uptake was measured for depolarized and nondepolarized synaptosomes (65mM and 5mM KCl, respectively) and net calcium uptake was calculated as the difference. Non-depolarized and depolarized synaptosomes from each of the brain regions were treated with 10μM D-Ala, D-Leu-enkephalin (DADLE). The calcium uptake of the DADLE-treated synaptosomes was compared to the calcium uptake of a matched control. No significant difference of the mean net calcium uptake was observed for the synaptosomes of any of the brain regions though individual experiments showed increases. A previous report (E. Barr and S.W. Leslie Fed. Proc. 43:968, 1984) showed significant increases of mean calcium uptake by hyppocampal and striatal synaptosomes treated with 10nM DADLE and dynorphin (A1-17). These differences are discussed with respect to the actions of various concentrations of opioid peptides. Supported by NIAAA AA05809 and RSOA AA00044 to SWL.

324.7 DIFFERENTIAL EFFECTS OF MET-ENKEPHALIN ON HIPPOCAMPAL CA1 COMPLEX-SPIKE AND THETA CELLS. K.Pang* and G.Rose, Dept. of Pharmacology, UCHSC and Medical Research, VAMC, Denver, CO.

Previous studies have demonstrated that opiates cause pronounced excitation of hippocampal pyramidal cells, but the mechanism by which this excitation is achieved is still unknown. One hypothesis, termed the disinhibition hypothesis, suggests that opiates do not directly excite hippocampal pyramidal cells, but instead act indirectly by suppressing the firing of local inhibitory interneurons.

Previous electrophysiological work has identified two neuronal types in area CA1 of the hippocampus: complex-spike (CS) cells and theta cells. These two neuronal types may be distinguished from each other by their unfiltered extracellular action potential durations. In area CA1, CS cells are generally considered to be the pyramidal cells, while theta cells are thought to be interneurons.

In the present study the effects of local application of methionine enkephalin (met-enk) were studied on CS cells and theta cells in area CA1 of the hippocampus in an attempt to provide additional support for the disinhibition hypothesis. Met-enk (10^{-5}M) and the opiate antagonist naloxone (10^{-4}M) were locally applied to hippocampal CS cells and theta cells by pressure ejection in anesthetized rats and the effects on spontaneous firing rate were determined. As has been previously reported, the major effect of met-enk on CS cells was to increase the spontaneous firing rate (27 of 31 cells; 3 neurons were depressed and 1 showed a biphasic response). In contrast, most theta cells were depressed (26 of 32 cells; 3 neurons were excited and 3 showed biphasic responses). Although naloxone application did not appreciably alter the spike height of CS cells, it did produce frequent decreases in the spike height of theta cells. Consequently, the studies involving naloxone antagonism of the theta cell response to met-enk were greatly complicated, but in those cases where a clear effect could be discerned, naloxone antagonized the met-enk induced response in 4 of 5 theta cells and 7 of 8 CS cells.

These results demonstrate that local application of met-enk increases the spontaneous firing rate of CS cells and depresses the spontaneous activity of theta cells in an opiate-specific manner. The findings of this study support the hypothesis of disinhibition as a mechanism of opiate action in the hippocampus. In addition, these results also provide additional evidence that the theta cells are the interneurons in area CA1 of the hippocampus.

324.8 INTERLEUKIN 1 INTERACTS WITH OPIOID BINDING SITES. M.S. Ahmed*, J. Llanos-Q.*, and C.M. Blatteis. Univ. of Tenn. Ctr. Hlth. Sci., Memphis, TN 38163.

Interleukin 1 (IL1) is a macrophage-derived monokine which signals neurons in the preoptic-anterior hypothalamus (POAH) to initiate fever and various other, associated, nonfebrile host-defense responses, collectively termed the acute-phase reaction. It is not yet clear whether IL1 acts directly or through the release of mediators. Recently, increases in cerebrospinal fluid and hypothalamic levels of β-endorphin (BE) have been reported during endotoxin (LPS)- and IL1-induced fevers; BE and certain other opioids are hyperthermogenic when microinjected into the POAH. However, naloxone does not prevent the febrile responses to LPS and IL1. Similarly, antipyretics do not antagonize the hyperthermia induced by centrally administered opioids. Might the opioid peptides, therefore, modulate the nonfebrile actions of IL1? To test this possibility, we determined whether IL1 affects the specific binding of opioid agonists in the brain. Accordingly, we assayed the possible competition of purified human IL1 with μ (^{3}H-dihydromorphine, DHM) and δ (^{3}H-2-D-alanine-5-L-methionine, DAME) radioligands in different guinea pig brain regions, using P2 opiate receptor-enriched membrane preparations. IL1 inhibited DHM binding, in order of decreasing potency, in the pons-medulla, hypothalamus-midbrain, and cortex. On the other hand, IL1 attenuated DAME binding most potently in the cortex and least potently in the hypothalamus-midbrain. Similar results were obtained with homologous, crude IL1. LPS (S. enteritidis suspended in pyrogen-free saline, in the concentration range from 4 to 40 ng/ml of binding assay medium) did not inhibit the binding of either opioid ligand to any of the brain regions. These results suggest that IL1 may interact with the opiate system in the brain. It appears, moreover, that such interactions may not be limited to the hypothalamus alone, the primary site of the febrigenic action of IL1.

324.9 THE OPIATE ACTIVATED POTASSIUM CONDUCTANCE IN LOCUS COERULEUS NEURONES. J.T. Williams and R.A. North. Neuropharmacology Laboratory, M.I.T., 56-245, Cambridge, MA 02139, U.S.A.

Intracellular recordings were made from locus coeruleus (LC) neurones in a slice preparation of rat brain. Drugs were applied by superfusion and by pressure ejection from a pipette placed in the superfusing solution 200 - 400 μm from the neurone. Opiates caused an outward current which was measured with a single electrode voltage clamp amplifier (Axoclamp II). The amplitude of the outward current was dependent on the concentration of agonist applied, was reproducible among cells for any given agonist and had a maximum amplitude of about 250 pA (at -60 mV; this is equivalent to a maximum opiate conductance of 6 nS). The conductance increase produced by [Met^5]-enkephalin, [D-Ala^2, D-Leu^5]-enkephalin or normorphine was voltage independent from -60 to -120 mV but declined sharply at potentials less negative than -50 mV. Agents which decreased potassium conductance also reversibly decreased the amplitude of the opiate induced outward current. The most selective of these agents were quinine (50 μM - 1 mM), barium (30 μM - 2 mM) and rubidium substitution (for potassium). Decreasing calcium entry by reducing extracellular calcium or addition of cobalt or magnesium had no immediate effect on the opiate induced outward current but after 15-20 min often reduced the amplitude of the opiate current. Increasing the extracellular calcium content had no effect on the opiate conductance. The voltage dependence and sensitivity to quinine and barium of the outward current caused by opiates suggest that opiates increase a potassium conductance which is also activated by intracellular calcium ions. The increase in potassium conductance accounts quantitatively for the hyperpolarizations caused by μ-receptor agonists. It leads both to an inhibition of action potential generation and a reduction in noradrenaline release from LC neurones.

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324.10 MET-ENKEPHALIN: DIFFERENTIAL EFFECTS IN THE HIPPOCAMPUS. L. Cahill*, H. Haigler, and R. Kochman (SPON: J. Bloss). Searle Research & Devlopment, 4901 Searle Parkway, Skokie, IL 60077.

Met-Enkephalin (ME) has been reported to produce an excitatory effect on neuronal firing when administered microiontophoretically in the hippocampus, but there was no reported histological confirmation of the recording sites (Zigelgansberger, W. et al. Science 205:415, 1979). We report here that the response to ME depends on the area of the hippocampus from which the recording was obtained.

The experiments were carried out as follows: Fisher 344 rats (350-400 gms) were anesthetized with chloral hydrate (CH) (400 mpk) administered i.p.; anesthesia was maintained by supplemental doses of CH. Microiontophoretic experiments were carried out using the techniques described previously (Hosford, D. and Haigler, H., J. Pharmacol. Exp. Therap. 213:355, 1980). When presumed hippocampal neuronal activity was encountered (i.e., negative action potentials that usually occurred in bursts of decreasing amplitude) the response to varying ejection currents of both ACh and ME were obtained. Drugs were ejected by a positive direct current (10-100 nA) applied to the drug barrel for a period of 50 seconds. At the end of an experiment fast green (FG) was ejected electrophoretically from the recording barrel, which was filled with 2M saline saturated with FG. The FG mark served as a reference point to localize the recording sites. The results are summarized below:

Location in Hippocampus	N	Increase N (%)	Decrease N (%)	No Effect N (%)
CA1	32	5 (16)	3 (9)	24 (75)
CA2,3,4	20	18 (90)	0	2 (10)

Note: Responsive and non-responsive neurons were found using the same micropipette

ACh increased firing of all of the above neurons indicating that the differential effect of ME on firing rates was not artefactual. These data may account for the variation in reports of percentage of hippocampal pyramidal cell excited by ME. These data also indicate that ME does not have a uniform effect on neurons in the hippocampus. The functional significance of this observation remains to be determined.

324.11 ALTERATIONS OF DYNORPHIN-LIKE IMMUNOREACTIVITY IN THE RAT BRAIN AFTER REPEATED ELECTROCONVULSIVE SHOCKS. T. Kanamatsu*, J.F. McGinty and J.S. Hong.Lab. of Behavioral & Neurological Toxicology, NIEHS/NIH, Research Triangle Park, NC 27709. Dept. of Anatomy, Sch. of Med., East Carolina Univ., Greenville, NC 27834.

It has been suggested that endogenous opioid peptides may mediate electroconvulsive shock (ECS)- elicited behavioral alterations, such as analgesia, retrograde amnesia, changes in seizure threshold or posticta1 depression. This notion was supported by our previous report that repeated electroconvulsive shocks (ECSs) increase the [Met^5]-enkephalin-like immunoreactivity (ME-LI) in certain limbic areas of the rat brain (Hong et al., Brain Res., 177: 273, 1979). Our recent study using in vitro cell free translation or blot hybridization to estimate the level of mRNA coding for preproenkephalin A suggested that the increase in hypothalamic ME-LI after repeated ECSs is due to an increase in biosynthesis (Yoshikawa et al., 9th IUPHAR Program 1984). These observations further support the possibility that an increase in enkephalinergic neuronal activity may be related to ECS-elicited phenomena. In an attempt to examine whether the other opioid peptides may also participate in the actions of ECS, we measured dynorphin A(1-8)-like immunoreactivity (DN-LI) in various rat brain regions after repeated ECSs. Ten daily ECSs caused an increase in DN-LI in hypothalamus (50%), caudate nucleus (30%), septum (30%) but no significant change was found in the frontal cortex and the neurointermediate lobe of the pituitary. However, the most prominent effect of repeated ECSs on the dynorphin system was in the hippocampus. A 70% decrease of DN-LI was found in the hippocampus after 10 daily ECSs. Detailed time course studies revealed that a single shock failed to alter the hippocampal DN-LI. A 30% decrease in DN-LI was found 24 h after 3 consecutive daily shocks. The maximal decrease (70%) was reached after 6 daily ECSs. The level of DN-LI in the hippocampus remained lower than the control value 4, 7 and 14 days after the secession of 6 daily ECSs (50%, 70%, and 80% of control value respectively). It is interesting to note that 10 daily ECSs caused a modest but significant increase (40%) of ME-LI in the hippocampus. Furthermore, the immunocyto-chemical studies confirmed the changes of ME-LI and DN-LI at different time points after ECS. This study plus our previous results suggest that alterations in opioid peptides in the limbic system may contribute to the behavioral changes observed after repeated ECS exposure.

324.12 IMMUNOCYTOCHEMISTRY SPECIFIES THE DYNORPHIN AND ENKEPHALIN CONTAINING NEURONS INVOLVED IN ELECTROCONVULSIVE SHOCK EFFECTS. J.F. McGinty, T. Kanamatsu*, and J.S. Hong. Dept. of Anatomy, East Carolina Univ. Sch. of Med., Greenville, NC 27834 and Lab. Behav. Neurol. Toxicol., NIEHS/NIH, Research Triangle Park, NC 27834.

Repeated electroconvulsive shock (ECS) treatments increase the concentration of dynorphin-like immunoreactivity (DN-LI) and [met^5]-enkephalin-like immunoreactivity (ME-LI) in the hypothalamus, caudate, and septum whereas the concentration of DN-LI decreases and ME-LI increases in rat hippocampus as measured by radioimmunoassay (RIA) (see Kanamatsu and Hong, this volume). To evaluate cellular integrity after repeated ECS and to elucidate the specific opioid pathways involved in these post-ECS changes, we conducted parallel immunocytochemical studies in rats treated identically as those treated with ECS for RIA measurements.

Rats were perfused and brains were prepared for immunocytochemistry (ICC) as described (McGinty, et al. PNAS 80:589, 1983). Frozen sections were incubated with antisera to dynorphin-A (1-17) (provided by L. Terenius, Uppsala, Sweden) or [leu^5]-enkephalin (LE) (provided by R.J. Miller, U. Chicago) followed by avidin-biotin-peroxidase immunoreagents. One day after six or ten daily ECS treatments, the intensity of DN-LI and LE-LI in medial basal hypothalamus, striatonigial (DN-LI) and striatopallidal (LE-LI) pathways, cells and fibers of the central nucleus of amygdala, the lateral septum, and ventral striatum (including the olfactory tubercle) was greater than in control brains. In the hippocampal formation, a marked decrease in DN-LI and LE-LI occurred in mossy fibers whereas a marked increase in LE-LI occurred in the perforant pathway originating in entorhinal cortex. The dentate granule cells, from which mossy fibers arise, appeared intact and healthy after repeated ECS treatments. A normal intensity of DN-LI and LE-LI was observed in all areas including hippocampus 14 days after secession of 6 daily ECS treatments. These data indicate that widespread opioid peptidergic pathways in the CNS metabolically react to repeated ECS treatments. In the hippocampus, the DN-LI contained in the granule cell-mossy fiber system is depleted probably as the result of intense stimulation by the presynaptic enkephalin-containing perforant pathway. Thus, physiological distinctions can be made between neurons containing these two opioid peptide families as a result of seizures. Supported by NS20451 (J.F.M.)

324.13 THE EFFECT OF MORPHINE ON BASAL RELEASE OF PROLACTIN IN LACTATING RATS. J. Rabii, P. Safier*, L. Grandison. Department of Biological Sciences, Rutgers University and Department of Physiology, UMDNJ, Piscataway, N.J. 08854.

The administration of a single injection of morphine causes a significant increase in circulating prolactin levels in male and non-lactating female rats. One of the mechanisms involved in opiate induced prolactin release is believed to be the inhibition of the tuberoinfundibular dopaminergic neurons. Recently, a lack of response of tuberoinfundibular neurons to prolactin feedback has been reported in lactating rats (Demarest et al., Neuroendo. 36:130, 1983). We have examined the effect of a single injection of morphine on basal levels of prolactin in lactating rats. Female rats, between four and ten days post-partum, were used in this study. Each rat was fitted with an indwelling venous cannula one day prior to the experiment. On the day of the experiment, dams were separated from their pups and two hours later were divided into three treatment groups. Two groups received morphine sulfate, one at a 10 mg/kg and the other at a 15 mg/kg dose (subcutaneously). The third group formed the saline controls. Blood samples were removed via the jugular cannulae prior to and at forty five minutes after morphine injection. In contrast to the stimulatory effects of morphine sulfate on prolactin release in male and non-lactating female rats, the administration of either dose of the opiate during lactation was ineffective in altering the circulating levels of this hormone. These results indicate that the tuberoinfundibular dopaminergic neurons, which are believed to be primarily involved in the maintenance of low prolactin levels during pup separation, are not responsive to opiates during this period. (Supported in part by NIH grant DA02227 and by grants from Charles and Johanna Busch Memorial Fund and Rutgers University Office of Research and Sponsored Programs.)

324.14 ALPHA-INTERFERON MODIFIES THE CHRONIC BUT NOT THE ACUTE MORPHINE EFFECTS, N. Dafny and C. Reyes-Vazquez, Neurobiology Department, University of Texas Medical School at Houston, Houston, Texas 77025, and Depto. Fisiologia, Fac. Med. UNAM, Mexico 04510.

The essential mechanisms underlying morphine (MOR) tolerance and physical dependence are not known. Changes in protein synthesis or in the immune system had been hypothesized to explain the chronic effects of opiates. Interferon (IFN) has antiproliferative and immunomodulating actions. The present experiments were initiated to study the effects of IFN on chronic and acute actions of MOR. Rats were made MOR-dependent by subcutaneous implantation of one MOR pellet containing 75 mg MOR base; 72 hours later, each animal was injected with naloxone (NAL) (1 mg/kg) to assess the degree of dependence as measured by the observed abstinence syndrome. Seven behavioral measurements were scored: wet dog shakes, stool discharges, teeth chattering, motor activity, cage exploration activity, scream-to-touch response and diarrhea. IFN was injected (150 I.U./g b.w.; ip) 1 hour before MOR pellet implantation (day 1) or 1 hour before NAL injection 71 hours after MOR pellet implantation (day 3). In both experiments it was found that IFN altered significantly the NAL induced abstinence syndrome. In addition, in other experiments the effect of MOR injection (5 mg/kg, ip) on rectal temperature and on the tail flick analgesic test was studied before and after IFN injection. It became evident that alpha IFN treatment did not modify normal temperature and did not cause analgesia as well as not modified the acute effects of MOR on rectal temperature and on the tail flick analgesia test. In conclusion, our experiments demonstrate that alpha IFN modifies only the chronic effects of MOR whereas the MOR acute effects remained unaffected by alpha IFN treatments.

324.15 CYCLIC AMP OR FORSKOLIN PRODUCES RAPID "TOLERANCE" TO THE DEPRESSANT EFFECTS OF OPIATES ON SENSORY-EVOKED DORSAL-HORN RESPONSES IN SPINAL CORD-DORSAL ROOT GANGLION (DRG) EXPLANTS S.M. Crain, B. Crain* and E.R. Peterson*, Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461

Exposure of fetal mouse cord-DRG explants to morphine (>0.1μM) results in naloxone-reversible, dose-dependent depression of DRG-evoked dorsal-horn synaptic-network responses within a few min (Crain et al'77). After chronic opiate exposure (1μM) for 2-3 days, these dorsal cord responses recover and can then occur even in >10μM morphine (Crain et al'79). In the present study, when naive explants were treated with forskolin (10-50μM)-- a selective activator of adenylate cyclase(AC)(Seamon & Daly'81)--for 10-30 min prior to and during exposure to 0.3μM morphine, the usual depressant effects on dorsal-horn responses often failed to occur (10-30 min tests). Dibutyryl cAMP (10mM) or the more potent analog, dioctanoyl cAMP(0.1mM), produced a similar degree of "tolerance" to opiates as 10μM forskolin. With high levels of forskolin (50μM), even concentrations of morphine up to 10μM were far less effective in depressing cord responses. These anti-opiate effects of exogenous cAMP and forskolin are probably both mediated by increases in intracellular cAMP. The onset of opioid-"tolerance" in cAMP- or forskolin-treated cord-DRG explants provides the first electrophysiologic support for the hypothesis that neurons may develop tolerance/dependence during chronic opioid exposure by a compensatory enhancement of their AC/cAMP system following initial opioid-depression of AC activity (rev. by Collier, Nature'80). Previous evidence relied primarily on behavioral tests (Ho et al,'73; Collier et al,'75) and biochemical analyses in cell cultures (Sharma et al,'75). It will be of interest to determine if dorsal-horn tissues of cord-DRG explants do, in fact, develop increased AC/cAMP levels as they become tolerant during chronic exposure to opiates.

The ionic mechanisms by which increased cAMP levels antagonize opiate depressant effects are not known. Opioid "tolerance" also occurs during acute exposure of cord-DRG explants to 4-aminopyridine(4-AP)(Crain et al, Life Sci.'82). Whereas 4-AP potentiates synaptic transmission by depressing K^+ conductance and thereby enhancing Ca^{++} influx through voltage-dependent Ca^{++} channels, we postulate that cAMP may reverse opiate-induced interference with AC/cAMP-regulated phosphorylation-dependent gates in the same (e.g. Reuter'79) or other channels in these neurons.

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324.16 OPIOID REGULATION OF NEUROTUMOR CELL GROWTH *IN VITRO*. P.J. McLaughlin and I.S. Zagon. Dept. Anatomy, The M.S. Hershey Medical Center, Hershey, Pennsylvania 17033.

Exogenous and endogenous opioids have been shown to modulate the growth of developing nervous tissue in both normal and abnormal neural models. Opioid agonists and antagonists affect the course of neuroblastoma (NB) in mice inoculated with these tumor cells. In addition, normal growth mechanisms can be altered by opioid exposure. Clinical models of offspring maternally exposed to opioid agonists, as well as laboratory studies on perinatal administration of agonists and antagonists, have indicated an active role for opioids in somatic and neurobiological development. In the present study we investigated the effects of opioids on cellular events under *in vitro* conditions, thereby eliminating many confounding influences present in intact biological systems. Cultures of S20Y NB cells were treated daily beginning 24 hr after seeding with heroin at concentrations of 10^{-2}M to 10^{-8}M; control cultures received equal volumes of sterile water. Growth curves were constructed at various points 12 to 84 hr following drug administration. The addition of heroin to cultures resulted in a decrease in cell number in a dose-dependent manner. Retarded cell division was confirmed by thymidine incorporation studies and analyses of mitotic figures. Morphological differentiation also was noted to be decreased in drug-treated cultures, but the effect was not necessarily dependent on heroin dosage. The action of heroin in perturbing cell growth was blocked by concomitant administration of an opiate antagonist, naloxone. The stereo-specific effects of opiate agonists were investigated by treating logarithmically growing cultures with equimolar concentrations of levorphanol or dextrorphan. Levorphanol-exposed S20Y NB cell cultures were growth inhibited in a fashion similar to that observed when heroin was applied, whereas dextrorphan had little effect on cell growth. The results of this study demonstrate that opioids alter growth processes of neurotumor cells under *in vitro* conditions. The growth inhibition following agonist treatment appears to be dose-dependent, stereospecific, and naloxone-reversible, with the locus of action postulated to involve the opiate receptor. These data support the contention that, in addition to a host of functions in adult organisms, endogenous opioid systems play an important role in developmental events.

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324.17 MORPHINE PELLETS LOWER THE HYPOTHALAMIC CONTENT OF PROOPIOMELANOCORTIN mRNA BUT NOT THAT OF PROENKEPHALIN mRNA. I. Mocchetti*, O. Giorgi, J. P. Schwartz, W. Fratta and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Morphine dependence occurs without changes in brain opiate receptors (Bmax or K_D) or in CNS content of opioid peptides. It is not known whether the dynamic state of brain opioid peptides changes. Since the opioid peptides are synthesized as part of high molecular weight proteins which function as precursors, we have used specific cDNA probes in a hybridization analysis of the mRNAs for these precursors. In order to clarify whether a change in opioid peptide synthesis participates in morphine tolerance, we have estimated the dynamic state of endorphin and enkephalin by measuring opioid peptide steady state concentrations and specific mRNA contents in various brain areas of morphine-tolerant and withdrawn rats.

To induce tolerance, slow-release morphine pellets (75 mg each) were implanted in rats for 5 or 10 days; to precipitate the withdrawal syndrome, one group of animals received a single injection of naloxone (1 mg/kg) 5 days after implantation, and was sacrificed ½ hour later.

Proenkephalin (PE) and proopiomelanocortin (POMC) mRNAs were analyzed by northern blot technique in striatum, hypothalamus, midbrain, brain stem and spinal cord. The content of PE mRNA and enkephalins did not change in brain structures of rats killed 5 or 10 days after implantation or during withdrawal. POMC mRNA was detectable only in hypothalamus and midbrain. We observed that in the hypothalamus of rats implanted with morphine pellets POMC mRNA content was decreased by 45% and this decrease was partially reversed by a single injection of naloxone. No change was detected in β-endorphin levels.

Our results thus suggest that during tolerance there might be a decrease of hypothalamic β-endorphin turnover which is partially reversed during withdrawal.

325.1 SEIZURE-PRODUCING TREATMENTS RESULT IN INCREASED ENKEPHALIN AND DECREASED CHOLECYSTOKININ IMMUNOREACTIVITY IN MOSSY FIBERS. C.M.Gall. Dept. of Anatomy, University of California, Irvine, CA 92717.

Two hippocampal seizure-producing treatments, intraventricular kainic acid administration and discrete lesions of the hilus induce an increase in the amount of enkephalin-like immunoreactivity in the rat hippocampal mossy fiber system. The present experiments analyzed the effect of these treatments on the level of enkephalin-like (ENK), dynorphin-like (DYN) and cholecystokinin-like (CCK) immunoreactivity in the mossy fiber system of the mouse hippocampus.

Adult Swiss Webster mice were sacrificed 1) without experimental treatment, 2) four days after the placement of a small unilateral electrolytic lesion in the hilus of the dentate gyrus, or 3) four days after intraventricular administration of 0.15 to 0.25 ug kainic acid. All surgery was conducted under ketamine/xylazine anesthesia. Separate tissue sections were processed for the immunocytochemical localization of enkephalin, dynorphin A, dynorphin B, or cholecystokinin-8 using the peroxidase antiperoxidase technique.

In agreement with previous studies on the rat, optimally placed hilus lesions caused a dramatic, bilateral increase in mossy fiber ENK relative to the untreated mouse. Kainic acid induced a similar increase. In contrast, the same hilar lesion cases exhibited a striking decrease in mossy fiber CCK relative to controls. The magnitude of the decrease in CCK corresponded with the magnitude of the increase of ENK within a given animal. Animals with the largest ENK increase exhibited a virtually complete depletion of mossy fiber CCK. Following kainic acid administration a more modest but definite decrease in mossy fiber CCK was seen. The CCK staining of the dentate gyrus inner molecular layer was not notably affected by the hilus lesion but appeared reduced with higher kainic acid doses. Finally, neither hilar lesions nor kainic acid administration was found to substantially influence the level of mossy fiber DYN. These data suggest that seizures have large and opposite influences on the amount of CCK and ENK in the hippocampal mossy fibers. It is not unlikely that the marked shift in the relative amounts of these peptides contributes to changes in the physiological activity within hippocampus from the pre- to post-seizure interval. (Supported by NSF grant BNS82-00319 and an Alfred P. Sloan Fellowship.)

325.2 ANALGESIA AND TOLERANCE FOLLOWING CONTINUOUS INTRATHECAL (IT) INFUSION OF MORPHINE (MOR) AND NOREPINEPHRINE (NE) VIA MINI-OSMOTIC PUMPS. C.W. Loomis*, B. Milne*, F. Cervenko*, and K. Jhamandas*. (SPON: J.V. Milligan). Departments of Anesthesia and Pharmacology and Toxicology, Queen's University, Kingston, Canada K7L 3N6.

Opiates and α-adrenergic agonists produce intense analgesia following IT or epidural administration by interaction with spinal opiate and α-receptors, respectively. A limiting factor in the chronic use of opiate analgesics is the development of tolerance. The objectives of this study were: (A) to compare the characteristics of MOR- and NE-induced analgesia and tolerance following continuous IT administration; and (B) to determine if there is cross tolerance to NE following continuous IT administration of MOR. Male, Sprague-Dawley rats (250-350g) were implanted with a polyethylene catheter in the subarachnoid space of the spinal cord. After recovery (24h), a mini-osmotic pump (ALZET 2001; infusion rate 1 µl/h) was connected to the IT catheter and implanted s.c. (day 0). For expt. A, rats received either normal saline, MOR (10 µg/µl) or NE (15 µg/µl) for 7 days. For expt. B, rats received either normal saline or MOR (10 µg/µl) for 5 days; all animals were then given NE (15 µg/µl) for 7 days. Analgesia was assessed daily using the tail-flick test. Body weight and behaviour were also assessed during the treatment. A significant analgesic effect was observed on day 1 of IT MOR and maximum analgesia was observed on day 3. Tolerance was apparent by day 5 but the tail-flick latency did not return to control until day 7. For IT NE, maximum analgesia was observed on day 1 and the tail-flick latency returned to control on day 3. No significant analgesia was observed with IT saline. None of the treatments influenced body weight. In animals treated with IT MOR for 5 days, the action of NE was significantly attenuated when compared with IT saline treated animals. The tolerance to NE and the apparent cross tolerance of NE to MOR was not due to the oxidation of NE in the pump. This study shows that continuous IT infusion of MOR and NE produces significant analgesia. Tolerance develops earlier to NE as compared to MOR following IT administration and there appears to be cross tolerance between MOR and NE.

(Supported by the Medical Research Council of Canada)

325.3 SEPARATION OF MORPHINE ANALGESIA FROM PHYSICAL DEPENDENCE BY NALOXONAZINE. G.S.F. Ling, J. MacLeod,* S. Lee, S.H. Lockhart* and G. W. Pasternak, The Cotzias Laboratory of Neuro-Oncology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021

Naloxonazine is a long-acting mu_1 opioid binding site antagonist which blocks various opioid effects including analgesia and catatonia but not others such as respiratory depression. In this study, we examined the development of physical dependence following the intravenous administration of morphine (50 ug/kg/min) in groups of rats administered saline or naloxonazine (20 mg/kg, i.v.) 24 hours earlier. In contrast to the marked elevation in peak tail-flick latencies seen in the saline rats (8.7±0.4 sec; n=37) during the first 2 hours of the morphine infusion, naloxonazine pretreated rats exhibited a maximal tail-flick latency of only 3.9±0.3 sec (n=17). After 24 hours of continuous morphine infusion, the infusion was terminated and withdrawal was precipitated with naloxone (4 mg/kg, s.c.). Ten quantal and 5 graded signs were measured over the first hour and weight loss was assessed for 7 days. No significant difference in onset, incidence or severity of signs was noted between the saline (n=15) and naloxonazine (n=15) groups ($p > 0.05$) with the exceptions of salivation and penile discharge, which were significantly less in the naloxonazine group ($p < 0.05$). Body weight loss at 24 hours post-naloxone was identical for both groups. In conclusion, blockade of mu_1 sites with naloxonazine and its resultant antagonism of morphine analgesia had little effect upon the production of many of the signs of physical dependence. This raises the possibility that different receptors may mediate morphine analgesia and many of the signs of physical dependence.

325.4 ROLE OF CATECHOLAMINES IN TOLERANCE TO MORPHINE. Christina VanderWende, Stanley Bielen* and Marie T. Spoerlein. Dep't Pharmacol. & Tox., Rutgers University, P.O. Box 789, Piscataway, N.J. 08854.

VanderWende and Spoerlein (1973) reported that dopamine (DA) acts as an antagonist to morphine-induced analgesia. This coupled with the report that chronic morphine leads to increased turnover of DA (Clouet and Ratner, 1970) led to the suggestion that DA may be involved in the development of tolerance to morphine. In the present study, we examined the effect of depleting catecholamines (CAs) with alpha methyl-p-tyrosine (AMPT) on the development of tolerance to morphine in order to further assess this possibility.

Male, Swiss Webster mice were implanted with morphine pellets (75 mg) under light ether anesthesia to induce tolerance. AMPT (250 mg/Kg, ip, 1x daily) was administered starting the day prior to the pellet implant to reduce the CA levels. Seventy two hours after the pellet implant, the animals received a sc injection of morphine (12 mg/Kg). Thirty minutes following the morphine injection, the animals were tested for analgesia using the tail clamp procedure described previously (VanderWende and Spoerlein, 1973). Other animals receiving the chronic treatments were decapitated and the brains removed to determine DA and norepinephrine (NE) levels.

Animals implanted with morphine pellets alone showed no significant difference in whole brain levels of either DA or NE when compared to untreated controls. Chronic AMPT reduced the DA and NE levels by 30% and 42%, respectively, as compared to untreated controls. In the group receiving chronic AMPT + morphine pellet, the DA and NE levels were down by 36% and 38%, respectively, as compared to the pellet control group.

Chronic AMPT, itself, caused an analgesic response which was equivalent to morphine administered to naive control animals and partially (50%) reversed the tolerance induced by morphine pellet implantation. Reduction of tolerance to morphine was also apparent in the mortality rate. Although no mortality occurred in the morphine pellet control group, there was a 60% mortality rate in the pelleted + AMPT group. Only an occasional animal died in the AMPT control group suggesting tolerance to morphine mortality was altered. Further studies to distinguish between DA and NE are in progress.

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325.5 PREGNANCY-INDUCED ANALGESIA: EFFECTS OF ADRENALECTOMY AND GLUCOCORTICOID REPLACEMENT. S.A. Baron* and A.R. Gintzler. Department of Biochemistry, Downstate Medical Center, Brooklyn, N.Y. 11203.

It has been demonstrated that during pregnancy, rats show a progressive increase in jump threshold which is followed by an abrupt decrease within 24 hours after delivery. While it has also been established that this elevation in pain threshold is mediated by opioids, it is not clear which endorphin-containing system is involved in this phenomenon. Since the adrenal glands play a role in some forms of opioid-mediated stress analgesia and since they have been shown to contain enkephalins, the involvement of the adrenal glands in pregnancy-induced analgesia was investigated.

Rats were time-mated and subjected to bilateral adrenalectomy or sham-adrenalectomy on day 12 of pregnancy. The flinch-jump test was used as an index of pain threshold during pregnancy and the post-partum period.

The results indicated that adrenalectomy does not prevent the elevation in pain threshold observed during pregnancy. However, because adrenalectomized rats have high circulating levels of B-endorphin, the increased release of this peptide could have masked any attenuating effect of adrenalectomy on pain threshold. Therefore, in a subsequent study, adrenalectomized pregnant rats were treated with 0, 160 or 240 ug/ml of corticosterone in order to suppress pituitary function. The data indicate that, overall, the jump thresholds of corticosterone-treated adrenalectomized rats were not significantly different from those of saline-treated adrenalectomized or sham-adrenalectomized rats. The persistence of the analgesic response in these rats indicates that the post-adrenalectomy increase in plasma B-endorphin did not mask an attenuating effect of adrenal removal on pain threshold.

Taken together, these experiments provide the first demonstration that the adrenal glands do not play a critical role in the opioid analgesia observed during pregnancy in rats.

325.6 THE EFFECTS OF THE ACUTE ADMINISTRATION OF BUPRENORPHINE HYDROCHLORIDE ON THE RELEASE OF ANTERIOR PITUITARY HORMONES IN THE RAT. R.N. Pechnick*, R. George* and R. Poland* (SPON: I.J. Bak). Dept. of Pharmacology, UCLA and Dept. of Psychiatry, Harbor General Hospital, Los Angeles, CA 90024.

Buprenorphine hydrochloride (BUP), a mixed agonist-antagonist has been reported to be a potent analgesic in man that causes neither physical dependence nor dysphoria. The present study was conducted in order to characterize the anterior pituitary hormone release pattern observed following the systemic administration of BUP in order to determine whether it elicits a morphine-like (mu) or ethylketocyclazocine-like (kappa) (Pechnick et al., J.P.E.T., in press) release profile.

Male, Sprague-Dawley rats were housed under a 12/12 hour light-dark cycle for eight days. For three days prior to the experimental day the subjects were handled and given s.c. saline injections in order to habituate them to the experimental procedure. On the day of the experiment the rats (48 days old) were randomly assigned to control and treatment groups and received either the vehicle control or BUP (0.13, 1.32 or 6.60 mg/kg, s.c.). 30 minutes following injection trunk blood was obtained for measurement of corticosterone, GH, PRL, luteinizing hormone (LH) and thyroid stimulating hormone (TSH) via RIA. Nonparametric statistical analysis was performed using a multiple comparison method and the experimentwise error rate set at 0.05 for determining significant differences.

BUP administration did not induce statistically significant changes in the levels of corticosterone. There was a tendency for BUP initially to increase corticosterone release but with increased doses of drug this tendency disappeared. Serum levels of GH increased while PRL release showed a biphasic response pattern with significant elevation following the low dose and a significant decrease following the high dose. Serum LH levels were not changed. Serum TSH levels were initially significantly decreased but no change was seen after the highest dose. The results demonstrate the BUP elicits a morphine-like pattern of activity at lower doses but this pattern becomes ethylketocyclazocine-like as the dose is increased.

325.7 A ROLE OF MU_2 OPIOID RECEPTORS IN OPIOID RESPIRATORY DEPRESSION. K. Spiegel, G.S.F. Ling and G.W. Pasternak, The George C. Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021

Although blockade of mu_1 sites with naloxonazine markedly attenuates morphine analgesia, respiratory depression measured with arterial blood gases is unaffected. These studies suggest that respiratory depression in the rat does not involve the mu_1, high affinity, opioid binding site. To explore the potential role of mu_2 and delta receptors in the respiratory depression, we examined the effects of morphine and two opioid peptides, metkephamid and D-ala^2-D-leu^5-enkephalin (DADL), on arterial blood gases. Metkephamid is a unique opioid peptide with very similar affinities for the mu_2 and delta receptors. In contrast, morphine and DADL discriminate effectively between the two sites, morphine binding 9-fold more potently to mu_2 than to delta receptors and DADL binding to delta sites 6-fold more potently than mu_2. Dose-response relationships for analgesia were determined and equianalgesic doses of morphine (3.5 mg/kg iv) and metkephamid (8 mg/kg iv) were injected. Morphine and metkephamid had virtually identical respiratory effects: morphine produced a drop in pO_2 of 17.3 mm Hg and a rise in pCO_2 of 12.3 mm Hg, while metkephamid decreased pO_2 19.3 mm Hg and raised pCO_2 9.9 mm Hg. For comparison of morphine and DADL, drugs were given intracerebroventricularly (icv) via indwelling cannulae. Unlike metkephamid, DADL produced much less respiratory depression than morphine at equianalgesic doses. Morphine (10 ug, icv) caused a rise in arterial pCO_2 of 23.3 mm Hg while DADL increased pCO_2 by only 1.7 mm Hg. These results suggest a role for mu_2 rather than delta binding sites in opioid respiratory depression.

325.8 SPONTANEOUS MORPHINE WITHDRAWAL FROM THE SPINAL CORD AFTER C-1 SECTION IN DEPENDENT RATS. J.J. Buccafusco and D.C. Marshall (SPON: W.J. Jackson). Depts. of Pharmacology and Toxicology, and Psychiatry, Medical College of Georgia and V.A. Medical Center, Augusta, GA 30912.

The autonomic component of morphine withdrawal can be evaluated by measurement of the postwithdrawal increase in mean arterial pressure (MAP). In intact dependent rats postwithdrawal MAP may increase by as much as 15-23 mmHg after 7-14 hr, respectively, of morphine deprivation (Pharmacol. Biochem. Behav. 18:209, 1983). Our recent studies have demonstrated that the spinal cord is a potential site for mediating this response since, 1) intrathecal injection of naloxone to intact dependent rats or 2) i.v. injection of naloxone to spinal (C-1) transected rats both elicit a marked increase in MAP. The purpose of the present study was to determine whether a withdrawal associated pressor response associated with abrupt discontinuation of morphine administration could be obtained in dependent spinal transected rats. Rats were made dependent by via a chronic intraarterial infusion of morphine (35-100 mg/kg) over 5 days. Control rats received only saline infusion. On the day of the experiment morphine was discontinued and 6 hr later rats were anesthetized with halothane, artifically respirated and transected at the C-1 level. MAP was recorded from an indwelling arterial catheter 30 min following transection and continued for 60 min. MAP was evaluated as the peak increase over control (stabilized pressure just prior to first reading) and as the average of 12, 5 min consecutive readings (AMAP). By 30 min after transection MAP in morphine dependent (N=5) and saline (N=4) groups was, respectively, 84±8 and 67±6 mmHg. During the 60 min measurement period only a slight, non-significant peak increase in MAP (6±2 mmHg) and AMAP (4±2 mmHg) was observed in the saline group. Morphine dependent rats, however, exhibited a significant peak increase in MAP of 21±2 mmHg and AMAP increase of 14±1 mmHg. Intraarterial injection of naloxone (0.5 mg/kg) at the 60 min point elicited a second marked increase in MAP of 84±4 mmHg with only a 6±1 mmHg increase recorded in the saline group. The origin of this response was the cord itself since pithing the cord from C-1 to L-1 just after transection in dependent rats abolished the subsequent increases in MAP to both spontaneous and naloxone-precipitated withdrawal. Supported by the Medical Research Service of the V.A.

325.9 AMYGDALOID ALPHA-2 ADRENOCEPTORS MIGHT MODULATE OPIATE WITHDRAWAL. Jonathan E. Freedman and George K. Aghajanian, Depts. Psychiatry and Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06508.

The alpha-2 adrenergic agonist clonidine is clinically useful in reducing the symptoms of opiate withdrawal. We have examined responses of single amygdaloid neurons mediated by alpha-2 adrenoceptors and opiate receptors in the rat and looked for alpha-2-opiate interactions. Extracellular recordings were made in vivo using 5-barrel microelectrodes. Rats were anesthetized with chloral hydrate and cell firing rates were maintained at 50% of maximum by microiontophoresis of glutamate.

Clonidine, applied microiontophoretically or at 20-100 µg/kg iv, reduced cell firing. Microiontophoresis of norepinephrine also reduced firing, while the alpha-1 agonist phenylephrine had little or no effect. Responses were antagonized by idazoxan, applied microiontophoretically or at 80 µg/kg iv. (Idazoxan (RX 781094) is an alpha-2 selective antagonist which we found in previous studies in rat locus coeruleus and dorsal raphe to have an alpha-2 selectivity markedly superior to yohimbine, rauwolscine, or piperoxane.)

Microiontophoresis of morphine or d-ala, d-leu-enkephalin also reduced amygdala cell firing, as did morphine at 1-10 mg/kg iv. (In contrast, hippocampal neurons were activated or disinhibited by morphine, as described by others.) Naloxone at 50 µg/kg iv antagonized these responses. Microiontophoretic naloxone also antagonized responses, but additionally had a nonspecific local anesthetic-like suppressant effect. After chronic morphine treatment (75 mg pellet sc daily, 4 days) cells were found which were activated above baseline by naloxone at 50 µg/kg iv. This increase in firing would presumably accompany opiate withdrawal.

Cells were found which responded to microiontophoresis of both morphine and clonidine. Such cells were found most frequently in the nucleus medialis. Thus clonidine might exert its effect on opiate withdrawal by counteracting the withdrawal-induced increase in cell firing. Other laboratories have shown that: (i) the amygdala is an important brain region in mediating opiate withdrawal symptoms, (ii) clonidine retains its effects on withdrawal after lesions of the dorsal noradrenergic bundle, and (iii) some amygdaloid alpha-2 binding sites may be postsynaptic. Taking these results together with our own, amygdaloid neurons appear a possible site of action for clonidine mitigation of opiate withdrawal.

325.10 THE BEHAVIORAL EFFECTS OF SELECTED OPIATES AND PCP IN THE NON-DEPENDENT AND CYCLAZOCINE-DEPENDENT RHESUS MONKEY. J. Bergman*, J. Hassoun* and C.R. Schuster (SPON: P.B. Dews) Laboratory of Psychobiology, Harvard Medical School, Boston, U.S.A. (J.B.) and Department of Psychiatry, The University of Chicago, Chicago, U.S.A. (J.H. and C.R.S.)

The development of tolerance to the behavioral effects of cyclazocine (CYC) and of tolerance to those of ketocyclazocine (KC), phencyclidine (PCP), naloxone (NAL), and the (+) and (-) isomers of N-allylnormetazocine (NAMN) were studied in rhesus monkeys. Each daily session consisted of six repetitions of a cycle in which an 8-min timeout was followed by a 3-min period in which every 30th response produced a food pellet. On test days, cumulative doses of a drug or vehicle were injected i.v. in sequential timeouts. Initially, all drugs decreased responding in a dose-related manner. Daily administration of up to 11 mg/kg CYC led to at least a 10-30 fold rightward shift in the CYC and KC dose-effect functions and lesser rightward shifts in the PCP and (+)NAMN functions. The effects of NAL and (-)NAMN were generally unchanged. The rate-decreasing effects of KC, but not of the other compounds studied, appear to be primarily due to its effects at cyclazocine-sensitive sites of action.

325.11 THE EFFECTS OF A SPONTANEOUS SEIZURE ON OPIATE RECEPTOR BINDING IN THE SEIZURE SENSITIVE MONGOLIAN GERBIL. R.J. Lee*, P. Lomax and R.W. Olsen. Dept. of Pharmacology, UCLA School of Med., Los Angeles, CA 90024. J.K. Wamsley. Dept. of Psychiatry, Univ. of Utah School of Med., Salt Lake City, UT 84132.

In previous studies from our laboratories we have demonstrated that opioid peptides decrease the incidence and severity of spontaneous seizures in our epileptic (SS) strain of Mongolian gerbil. Opioids also appear to modulate the events which suppress further seizures in the post-ictal refractory period which follows a spontaneous seizure. Mild restraint of the animals, exposure to lower ambient temperatures or administration of ACTH and adrenal steroids can also change the incidence and pattern of spontaneous seizures in these animals. Thus, conditions which might alter the activity of the pituitary peptides in the CNS seem to change the seizure propensity in the SS gerbil, and endogenous opioids may play a role in suppressing the seizure diathesis in the immediate post-ictal period. The density of specific opiate receptor binding has been compared in several brain regions of SS gerbils, prior to and immediately following a spontaneous seizure, using cryostat sections of brains labeled with [^{3}H]-dihydromorphine subjected to autoradiography and analysed by microdensitometry. Brains from a non-seizing (SR) strain were also examined. Brains removed from SS gerbils before seizure demonstrated greater opiate binding in the superior colliculus (11%), periaqueductal gray (22%), substantia nigra (29%) and the dentate gyrus (12%) when compared to SR animals. When the brains of SS animals were examined immediately following a seizure there was a general decrease in binding density compared to the pre-ictal period; binding in the substantia nigra was 18% less, falling to a level the same as that in the SR strain. A tendency toward lower benzodiazepine and GABA receptor binding has been demonstrated in the brainstem of SS gerbils which increased following a seizure, particularly in the substantia nigra. These changes in various membrane receptors could underlie the abnormal epileptic state in the SS strain. It is not yet clear whether or not the change in opioid binding is due to alterations in the number of receptors.

325.12 A COMPARISON OF THE ANALGESIA, RESPIRATORY DEPRESSION, AND TASTE AVERSION OF PENTAZOCINE, MORPHINE AND BROMADOLINE (U-47931) IN THE RODENT. Peggy J. K. Dobry-Schreur, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Bromadoline maleate is an analgesic of the narcotic antagonist type, with a higher agonist/antagonist ratio than pentazocine's.

ANALGESIA: TAIL-SHOCK VOCALIZATION TEST. Rats were tested in an up-down titration method for the threshold tail-shock current to elicit vocalization. Bromadoline (B) was about as potent as codeine PO_4 (C) and pentazocine lactate (Talwin^R) (P); morphine SO_4 (M) was more potent. Naloxone HCl completely antagonized analgesia caused by B, C, and M, but not P. P was the only compound which antagonized M's analgesia.

ANALGESIA: ZINGERONE TEST. A drop of zingerone suspension was placed into one eye of a mouse. Immediate, continuous forepaw wipes at the face were counted. M was the most potent analgesic (ED_{50}=1.9 mg/kg s.c.); B maleate, C, and P were about equipotent (16, 15, and 13 mg/kg, respectively).

RESPIRATORY DEPRESSION. Blood was drawn anaerobically from rats with chronic aortic cannulas and analyzed within 4-6 min for pH, pCO_2, and pO_2 with an Instrumentation Laboratory Model 313 blood gas analyzer. M caused significant respiratory depression; arterial pH and pO_2 were decreased at 3-30 mg/kg; pCO_2 was increased at 10-30 mg/kg. B also caused respiratory depression, but only at 30 mg/kg. P slightly decreased pH at 10-30 mg/kg but did not alter pCO_2 or pO_2. Pre-injection control values for 48 rats were pH=7.49±0.005; pCO_2=25.4±0.6 mmHg; pO_2=82.1±1.6 mmHg.

TASTE AVERSION. Mice were offered 5% sucrose for 15 min on Days 1 and 2; drug was injected immediately afterward. On Day 4 mice were offered sucrose solution for 10 min, in the presence of water (only the consumption of sucrose solution was measured). All 4 compounds caused a dose-related taste aversion. The doses causing 25% and 50% decreases in sucrose drinking on Day 4 were: M 2.4 and 3.9 mg/kg; P 3.4 and 5.5; B 15 and 22; and C (in a slightly different protocol) 33 and 40.

Thus, in 2 tests of analgesia in the rat and mouse, B was about as potent as C and P, but less potent than M. B caused less respiratory depression than M and more than P. In taste aversion (presumed to be a measure of subjective effects), B was intermediate in potency between M and P on the one hand, and C on the other.

325.13 A CHOLINERGICALLY-MEDIATED AROUSAL EFFECT OF CODEINE: POSSIBLE EXPLANATION FOR ITS ANTINARCOLEPTIC ACTION. A. Horita*, M. A. Carino* and Y. S. Pae* (SPON: R.M.Quock). Depts. of Pharmacol. & Psychiatry, Univ. Wash. Sch. of Med., Seattle, WA 98195.

We reported earlier (Neuropharmacol. 22:1183, 1983) that intracerebroventricular (icv) injections of morphine to pentobarbital-anesthetized rabbits produced arousal and a shortening of the duration of anesthesia (analeptic effect) as determined by the recovery of the righting reflex. Intravenous morphine potentiated the depressant effect and caused lethal respiratory depression. Pretreatment with naltrexone prevented the latter effect and unmasked the analeptic property. We now report that codeine is also effective against various CNS depressant agents. One major difference between the codeine- and morphine-induced analeptic effect was that codeine produced analepsis upon iv admnistration and in the absence of naltrexone. Doses of 1-2 mg/kg codeine were effective in shortening the narcosis duration of ketamine and diazepam. When the codeine dose was increased to 10 mg/kg the analeptic effect was not longer evident, but reappeared when animals were pretreated with naltrexone. It is evident that at the higher doses the mu-receptor-mediated depressive action dominated, and when naltrexone blocked this effect the analeptic effect was unmasked. This effect produced by both morphine and codeine was completely blocked by atropine, but not by methylatropine, pretreatment. We conclude that codeine, like morphine, produces a cholinergically-mediated arousal effect. This response is more evident with codeine because of its much weaker mu-receptor mediated depressant action. We feel that this arousal action is responsible for the recently reported ameliorative effect of codeine in the treatment of human narcolepsy (Fry & Pressman, Neurol. 33 (suppl. 2):176, 1983).

325.14 SPINAL ANALGESIA WITH FENTANYL ALTERS LOCAL SPINAL BLOOD FLOW. G. Crosby. Dept. of Anaesthesia, Massachusetts General Hospital and Harvard Med. Sch., Boston MA 02114.

Spinal administration of narcotic analgesics produces marked segmental analgesia (Yaksh, T.L., Pain, 11:293, 1981) without, at least in the case of morphine, altering white matter spinal blood flow (SCBF) (Matsumiya, N., Dohi, S., Anesthesiology, 57:175, 1982). Inasmuch as more potent and lipid soluble narcotics might yield different results, the effect on local spinal blood flow (LSCBF) of fentanyl spinal analgesia was studied in conscious male Sprague-Dawley rats. Animals were prepared with a spinal subarachnoid catheter (Yaksh, T.L., Rudy, T.A., Physiol Behav, 17:1031, 1976) and 24-72 h later, under light halothane-nitrous oxide anesthesia, femoral artery and vein catheters. Rats were then allowed at least 3 h to recover from surgery and general anesthesia before spinal analgesia was induced. Four animals received an ED95 dose for spinal analgesia of fentanyl (3 μg) (Yaksh, T.L., Rudy, T.A., J Pharmacol Exp Ther, 202:411, 1977) and 10 μl of saline flush intrathecally; 6 control animals received only saline. The LSCBF of lumbar spinal cord was measured 10-15 min later with the autoradiographic iodo-[14C]antipyrine method (Sakurada, O., et al., Am J Physiol, 234:H59, 1978). Data were analyzed with a grouped t test. There were no statistically significant differences in blood pressure, arterial blood gases and pH, or temperature between the groups. Fentanyl produced 2-9% increases in spinal white matter blood flow, but only the 9% increase in ventral white matter was statistically significant ($P < 0.01$). In contrast, spinal gray matter blood flow was statistically significantly increased 20-50%. Flow increased least in laminae I-III (20%) and IV-VI (37%); 46-50% increases occurred in laminae VII, VIII, and IX. Like intrathecal morphine, fentanyl has little effect on spinal white matter flow. Perhaps intrathecal morphine also increases gray matter SCBF, but this has not been studied. Since naloxone reversibility of fentanyl's LSCBF effect has not yet been attempted, it is uncertain whether it is mediated by spinal opiate receptors. However, the fact that blood flow increases selectively in spinal gray matter makes a non-specific effect of fentanyl (e.g., direct vasodilatation) unlikely.

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325.15 DIFFERENTIAL EFFECTS OF MU, KAPPA AND DELTA AGONISTS ON MOTILITY OF THE CANINE SMALL INTESTINE EX VIVO. L.D. Hirning*, V.J. Hruby*, R. Hurst* and T.F. Burks, Departments of Pharmacology and Chemistry, University of Arizona, Tucson, Arizona 85724.

Opioids are known to produce phasic contractions of the canine small intestine in vivo or ex vivo. This contractile activity is believed to be responsible for the constipating effects of these agents in vivo. However, the receptor subtypes involved in the peripheral effects of opioids have not been fully explored. We have examined the actions of receptor selective opioids in an attempt to ascribe a receptor subtype to these effects. D-Ala^2-MePhe^4-Gly(ol) enkephalin (DAGO), D-Pen^2-L-Cys^5 cyclic enkephalin (DPLCE) and U-50,488H (trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolindinyl) cyclohexyl] benzeneacetamide methane sulfonate) were employed as the selective μ,δ and κ receptor agonists, respectively. The effects of these opioids were evaluated in the canine isolated intestine ex vivo. Segments of bowel were removed from anesthetized mongrel dogs (either sex, 15-40 kg.) and arterially perfused with warmed (37° C), oxygenated krebs-bicarbonate buffer. Contractile responses were recorded from a latex balloon secured within the lumen of the segment and measured as the peak in intraluminal pressure produced following intraarterial administration of the agonist. Dose-response lines were determined for each agonist and then repeated in the same segment in the presence of naloxone (0.1-3.0 μg/ml) in the perfusate. Both DAGO and DPLCE (0.2-25 μg i.a.) produced dose-related contractile activity of the isolated segments. In contrast, U-50,488H (0.1 - 100 μg i.a.) failed to produce motility of the intestine segments at any of the doses examined. Naloxone perfusion (100 ng/ml) of the intestine segments significantly inhibited the response to DAGO but did not affect those to DPLCE. The DPLCE-induced contractions were inhibited by naloxone but at concentrations 15-20 fold greater than those required to inhibit DAGO. The naloxone-resistant nature of δ-mediated responses has been previously reported for the mouse vas deferens. These data suggest that the contractile responses to opioids are mediated via μ and δ receptors in the dog intestine and that the κ receptor is not involved in the direct contractile responses induced by opioids. (Supported by USPHS grants DA02163 to TFB and NS19972 to VJH.)

325.16 MATERNAL-FETAL DISTRIBUTION OF OPIATES IN THE NEAR-TERM SHEEP AND RHESUS MONKEYS. M.S. Golub, J.H. Eisele, Jr.* J.H. Anderson*. California Primate Research Center, University of California, Davis, CA 95616.

Maternal-fetal distribution of the opiate agents morphine and alfentanil was studied in three anesthetized near-term sheep and rhesus monkeys. Blood samples were obtained from maternal and fetal arteries for radioimmunoassay 2, 5, 10, 30 and 60 min after drug administration from catheters placed under halothane anesthesia. Fetal-maternal ratios (F/M) for sheep increased from 0.04 to 0.47 during the first hour after bolus i.v. injection of 200 μg/kg morphine. However, in monkeys, F/M values were considerably higher, increasing from .27 to 1.85. Indeed, values over 1.00, indicating higher morphine concentrations in fetal than maternal plasma, were seen in two monkeys at 10 min and 60 min time points. For alfentanil, a more lipid soluble and protein bound drug, F/M values were similar in the two species ranging from 0.1 to 0.6 after injection of 250 μg/kg in sheep and 125 μg/kg in monkeys. The data also suggested that morphine was metabolized more rapidly in monkey dams than in ewes and that morphine glucuronide, the principle metabolite of morphine, was more readily distributed to monkey than sheep fetus. We conclude that opiate drugs with low lipid solubility and protein binding may transfer more readily from dam to fetus in primate than in ovine species near term. (Supported by GM 32920, RR00169 and Janssen Pharmaceutica).

325.17 HYPOTHALAMIC OPIOID PEPTIDE REGULATION OF BASAL AND STRESS-INDUCED CATECHOLAMINE SECRETION. J.A. Kiritsy-Roy* and G.R. Van Loon (SPON: J.A. Dougherty). VA Medical Center and Department of Medicine, University of Kentucky, Lexington, KY 40511.

Studies in this laboratory have demonstrated that central administration of opioid peptides elevates plasma catecholamine (CA) concentrations in rats. Since the paraventricular nucleus of the hypothalamus (PVN) contains a high level of opioid binding sites and is important for regulation of the sympathetic nervous system, we hypothesized that opioid peptides act in the PVN to modulate basal and stress-induced CA secretion.

Male Sprague-Dawley rats were surgically implanted with a guide cannula which terminated 2 mm above the right PVN and with a polyvinyl catheter in the left carotid artery for blood sampling and mean arterial pressure (MAP) and heart rate (HR) recording. Thirty minutes prior to the onset of drug infusion, a drug-filled inner cannula was lowered into the PVN through the guide cannula. Drugs were infused over 2 minutes in 200 nl of saline in unstressed, freely-moving rats. Plasma CA, MAP and HR were determined 5 min before, and 25 and 35 min after PVN microinjection of the mu-receptor agonist, [D-Ala^2,NMe-Phe^4,Gly^5-(ol)]enkephalin (DAGO) 0.01, 0.03 or 0.1 nmol, naloxone (NAL) 0.1 nmol, or saline. At 38 min, the animals were restrained in wire-mesh holders and CA, MAP and HR were measured 2 and 10 min later.

DAGO caused dose-related increases in NE (0.03-0.1 nmol, $p<0.05$), E (0.01-0.10 nmol, $p<0.05$) and HR (0.10 nmol, $p<0.05$). No apparent changes in MAP were seen at the times studied. Restraint stress elevated NE, E, MAP and HR. DAGO, 0.01 nmol but not higher doses, potentiated the E response to stress, without affecting the other stress responses. NAL alone had no effect on basal NE, E, MAP or HR, but NAL did enhance the E response to stress.

Thus, under basal conditions, a mu-receptor agonist acts in the PVN to stimulate central sympathetic outflow and increase plasma CA secretion. The opioid antagonist, NAL, does not appear to alter basal CA secretion. However, during restraint stress, NAL as well as a low dose of mu-agonist potentiate the stress-induced increases in plasma E. We conclude that opioid peptides act in PVN to increase plasma CA concentration, and that an endogenous opioid peptide may function in this brain site to regulate stress-induced CA secretion.

(Supported by the Veterans Administration and University of Kentucky Tobacco and Health Research Institute)

PEPTIDES: PHYSIOLOGICAL EFFECTS II

326.1 BIPHASIC RESPONSE OF A BURSTING NEURON TO FMRF-AMIDE. P.Ruben*, J.W.Johnson* and S.Thompson*. (SPON:C. Cohan) Hopkins Marine Station, Stanford University, Pacific Grove, Ca. 93950.

The cardio- and neuro-active peptide FMRF-amide has been shown to produce inhibition and excitation of spiking in molluscan neurons (Stone and Mayeri, Soc. Neurosci. Absts. 7:636, 1981). FMRF-amide has also been demonstrated to increase the delayed rectifying outward K+ current (IK) while decreasing the calcium-activated K+ current (IC) in Helix neurons (Cottrell, 1982, Nature 296:87-89).

We have studied the effects of FMRF-amide on the spiking activity and ionic currents of the neuron L6, a left upper quadrant burster in the Aplysia abdominal ganglion. Localized bath application of the peptide (10nM-10uM) produces a transient depolarization followed by a sustained hyperpolarization of L6. These effects are quickly reversible by washing with ASW. The biphasic response was studied using a two microelectrode voltage clamp. When the membrane potential was held at -40mV, FMRF-amide produced a transient inward current lasting about 5-10s, followed by a prolonged outward current which persisted until wash. A fast conductance measurement was used to determine the sign and reversal potentials of these currents (Johnson and Thompson, Soc. Neurosci. Absts. 9:1187, 1983). These measurements reveal that both phases are due to conductance increases. The reversal potential for the early, inward phase is about -20mV, suggestive of simultaneous inward and outward currents. The reversal potential for the late, outward phase was found to be close to the equilibrium potential for K+. When external Ca++ is replaced by Ni++ neither phase of the FMRF-amide response is diminished, suggesting that they are not Ca++ dependent conductances. The nature of these conductances will be discussed. Preliminary observations of FMRF-amide's effect on other membrane conductances indicate that, unlike in Helix neurons, neither IC nor IK are significantly altered.
Supported by the Alberta Heritage Foundation for Medical Research.

326.2 THYROTROPIN-RELEASING HORMONE INDUCES SPONTANEOUS BURSTING IN NEURONS OF THE NUCLEUS TRACTUS SOLITARIOUS. M. S. Dekin, G. B. Richerson*, and P. A. Getting, Department of Physiology and Biophysics, University of Iowa, Iowa City, Iowa 52242.

Thyrotropin-releasing hormone (TRH) increases respiratory rate and antagonizes the depressant effects of opiates and barbiturates on respiration (Hedner et al., Acta Physiol. Scand., 117; 1981). Its mechanism of action, however, is unknown. We have employed a brainstem slice preparation from guinea pigs to study the effects of TRH on neurons in the ventral region of the nucleus tractus solitarious (NTS). This area is a premotor integrating center for respiratory movements. In the slice preparation, NTS neurons fired non-rhythmically at a frequency of 1 to 2 spikes/sec (figure 1A). Following the addition of 200 to 500 nM TRH to the perfusion fluid these neurons developed a rhythmic oscillation in their membrane potential. The oscillations were voltage dependent, and disappeared when the membrane potential was current clamped at -70 mV. This voltage dependence indicated that the oscillation was not synaptically induced. The magnitude of the membrane potential oscillation increased with time and a bursting pattern was established (figure 1B). Each burst was followed by a depolarizing after-potential. Bursting occurred with a mean cycle period of 1.2 sec. The average firing rate within the burst was 25 Hz. These data indicate that TRH induced pacemaker properties in NTS neurons. There is evidence that the respiratory rhythm does not originate in the NTS. Our data, however, suggests that the NTS could be transformed into a rhythm generating locus in the presence of TRH.
(Supported by NIH grant HL 32336).

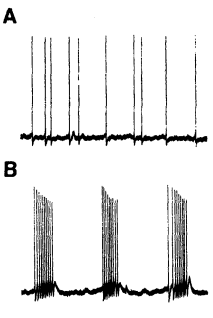

Figure 1. (A) Control record of non-rhythmic activity in a NTS neuron. Resting potential was -45 mV. (B) Bursting activity in same neuron after exposure to 400 nM TRH. Scale: 20 mV, 400 msec.

326.3 PEPTIDE MODULATION OF ACH CURRENTS IN AUTONOMIC NEURONS. L.W. Role. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Immunohistochemical techniques have revealed substance P (SP), enkephalin (ENK) and somatostatin (SOM) in preganglionic structures of the paravertebral sympathetic ganglia in the chicken (LaValley and Ho, J. Comp. Neurol. 213(4):406-413, 1983). Furthermore, the former two peptides have been demonstrated in most nerve terminals contacting the neurons of the avian ciliary ganglion (Erichsen et al., J. Neurosci. 2:994-1003, 1982). The possible presynaptic co-localization of these peptides with acetylcholine (ACh) led me to examine whether the physiological role of the peptides might be to modulate the action of the ACh on the postganglionic neurons. I have employed whole cell voltage and current clamp recording in in vitro lumbar sympathetic and ciliary ganglion neurons to address this question.

In both ciliary ganglion and sympathetic neurons SP (10 μM) causes a dramatic enhancement of the rate of ACh current decay in the absence of any direct effect on membrane potential, input resistance or voltage sensitive conductances (n=40). Examination of the steady state IV relationship for ACh currents evoked in the presence of SP reveals a 14-fold decrease in the slope conductance compared with control (Role, PNAS, in press). SOM (10-20 μM) had no detectable effect on ACh currents in sympathetic neurons. The ENK analog D-Ala^2-methionine-ENK produced a slight increase in the rate of ACh current decay in two of four sympathetic neurons tested.

Because SP markedly increases the rate of inward current decay in response to applied ACh, it is important to examine the effect of the peptide on synaptic transmission. Sympathetic neurons can be innervated in vitro using explants of dorsal spinal cord which contain the preganglionic column of Terni (L. Role and R. Hume, unpublished observation). The inward synaptic currents recorded in voltage clamped sympathetic neurons which have been innervated in this manner are probably cholinergic in that like applied ACh, (a) they have a reversal potential of -8 mV and (b) they are abolished by 10 μM curare. In preliminary experiments designed to examine the effect of the peptides on synaptic transmission, SP (10-20 μM) caused a statistically significant ($p<.001$ - $p<.1$) decrease in synaptic current duration in five of eight cells tested. These results indicate that SP may modulate ganglionic transmission by increasing the rate of ACh current decay.

326.4 CORTICOTROPIN-RELEASING FACTOR: ROLE IN CENTRAL NERVOUS SYSTEM REGULATION OF THE ADRENAL MEDULLA. M.R. Brown, L.A. Fisher, W.W. Vale and J.E. Rivier. Peptide Biology Lab, The Salk Institute, La Jolla, CA 92037.

Corticotropin-releasing factor (CRF) acts within the central nervous system (CNS) to elicit changes in autonomic nervous system activity similar to those changes produced by a variety of stressful stimuli, e.g., increased plasma concentrations of epinephrine and norepinephrine, and increased heart rate and mean arterial pressure. To examine the physiologic role of brain CRF in mediating stress-induced changes of autonomic nervous system function, we have carrried out experiments using a recently characterized CRF receptor antagonist. Peptides were administered intracisternally (ic) in lightly etherized rats, and bloods collected by cardiac puncture 20 or 40 min later. Plasma catecholamine concentrations were determined using a radioenzymatic assay.

CRF (1.5 nmoles, ic) produced a significant elevation of plasma epinephrine and norepinephrine levels. Simultaneous administration of [Glu^{27}]-CRF$^{10-41}$ (25 nmoles, ic) completely prevented this CRF-induced elevation of plasma catecholamine levels.

To determine if endogenous brain CRF might be involved in the regulation of the adrenal medulla or peripheral noradrenergic activity, the CRF receptor antagonist was administered to animals exposed to ether vapor or subjected to insulin-induced hypoglycemia. [Glu^{27}]-CRF$^{10-41}$ (2.5 and 25 nmoles, ic) produced a dose-dependent inhibition of plasma epinephrine levels but did not influence plasma norepinephrine levels following treatment with ether-vapor or insulin-induced hypoglycemia.

These results suggest that endogenous brain CRF may be involved in the physiologic regulation of adrenal epinephrine secretion.

326.5 EEG EFFECTS OF SUBCUTANEOUS AND INTRACEREBROVENTRICULAR INJECTIONS OF ARGININE VASOPRESSIN IN THE RAT. C.L. Ehlers*, T.K. Reed*, C. Lebrun*, and G.F. Koob (SPON: E.L.F. Battenberg). Division of Neurology and Neuroscience, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Several studies have suggested that arginine vasopressin (AVP) may act centrally to produce electrophysiological and behavioral effects. However, there are few reports of EEG effects of AVP in unanesthetized, awake animals. In the present study 43 albino rats were stereotaxically implanted with skull electrodes and were assigned to three experimental groups. The first group (n=14) received saline/6 μg AVP subcutaneously (S.C.), in a dose known to raise blood pressure. Rats in the second group (n=23) were implanted with intracerebroventricular (i.c.v) cannulae for central injections of either saline or behaviorally relevant doses of AVP (0.1, 0.5, or 1 ng). A third group of rats (n=6) were also implanted with i.c.v. cannulae, but were given three central injections of high (1.0 μg) multiple doses of AVP (purported to cause convulsions) spaced 2 and 5 days apart. Spectral analysis was utilized to uncover whether significant (Mann Whitney-U $p < .05$) amplitude or frequency characteristics of the EEG could be discerned. A significant dose related decrease in high frequency activity (16-32 Hz) was observed following i.c.v. injections of 0.1, 0.5 and 1 ng of AVP. In addition, at the 1 ng dose, significant decreases in the 2-8 Hz and 8-16 Hz ranges were also noted. Subcutaneous doses of AVP (6 μg/kg) produced some similarities to the 1 ng i.c.v. dose with decreases in the 2-4 Hz and 8-16 Hz range reaching significance. ICV administration of high (1.0 μg) doses of AVP produced an apparent decrease in exploratory behavior, associated with some EEG slow wave activity and a flattening of the amplitude of the signals. Subsequent ICV injections did not produce any additional EEG changes. However, a more potent behavioral effect was noted with some animals displaying barrel rolling or complete behavioral arrest. These studies suggest that single or multiple doses of AVP do not produce any EEG signs of epileptic seizures. In addition, the similarity in EEG activity between s.c. injections and the 1.0 ng i.c.v dose suggest a parallel mechanism for peripheral and central actions of AVP. However, the EEG effects seen following low-dose central injections (0.5, 0.1 ng) are supportive of a direct action of AVP on neural substrates of cognitive/behavioral activity. (Supported by NINCDS Grant 20912-01 and NSF grant INT 8215308 to GFK.)

326.6 EFFECT OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) ON THE NEURONE OF CAT PARASYMPATHETIC GANGLION. T. Akasu*, P. Shinnick-Gallagher and J.P. Gallagher. (SPON: P.M. Adams) Dept. of Pharmacology, The University of Texas Medical Branch, Galveston, TX 77550.

Intracellular and voltage-clamp recordings were made from the neurons of cat vesical parasympathetic ganglia (VPG). The application of vasoactive intestinal polypeptide (VIP) by pressure ejection (4-5 msec, 18-20 psi) through a micropipette placed near the recording electrode produces a long-lasting depolarizing response (1.5-3 min) in VPG neurons. The mean amplitude of VIP depolarization was 11.3 ± 4mV (n=35). The depolarizing effect of VIP was dose-dependent. The minimum effective concentration of VIP, 0.05μM, produced 0.5-2mV depolarizations. Apparent affinity constant (Km) estimated from the double reciprocal plot of dose-response relationship was 2.5μM. The VIP depolarization was frequently associated with action potentials in quiescent neurons and increased the frequency of the activity in spontaneously firing neurons. The VIP depolarization was associated with an increase in membrane resistance. The amplitude of VIP depolarization was decreased by membrane hyperpolarization and eventually reversed its polarity at -96.8 ± 11.2mV (n=4). The reversal potential estimated from V-I curve was -89.1 ± 8.3mV (n=11). An elevation of external K^+ depressed the VIP depolarization and lowering external K^+ increased the VIP depolarization. The reversal potential shifted to a more negative level in a low K^+ (0.5mM) solution, while it shifted to a more positive level in high K^+ (10mM) solution. Voltage-clamp recording showed that VIP (5μM) produced an inward current (0.83 ± 0.31nA; n=8) at the holding potential of -60mV. The VIP (5μM) current was accompanied by a decreased membrane conductance (2.8 ± 0.7nS; n=6). The reversal potential of VIP current was -105 ± 11.3 mV (n=3). VIP had no effect on the M current (Brown & Adams, Nature, 283, 673).

These results suggest that VIP depolarized the neurons of VPG by suppressing resting K^+ conductance. The significance of VIP receptors in VPG neurons is not known, but since VIP is present in afferents contained in the pelvic nerve and is present in some VPG neurons, it is possible that VIP may serve as an excitatory transmitter in a local reflex. Supported by NS16228.

326.7 THE EFFECTS OF PITUITARY NEUROPEPTIDES ON THERMOREGULATION AND FEVER IN THE CONSCIOUS RABBIT. A.M. Naylor*, W.D. Ruwe, W.L. Veale and M. Chrétien*. Department of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 4N1 and Protein and Pituitary Hormone Laboratory, Clinical Research Institute of Montreal, Montreal, Quebec H2W 1R7.

Arginine vasopressin (AVP) has been shown to exert marked thermoregulatory actions. Microinfusion of this peptide alters normal thermoregulatory function in the rat, whereas perfusion of AVP attenuates fever of bacterial origin in the sheep. This work was done to further characterize the actions of vasopressin on temperature regulation in the New Zealand White rabbit. In addition, human pituitary glycopeptide (HPGP), a postulated component of the precursor of neurophysin-arginine vasopressin, was examined for any similar actions in this species.

Stainless steel guide tubes were implanted above the ventral septal area and lateral cerebral ventricle (LCV) of male rabbits under pentobarbitol anaesthesia. AVP (0.626-6.5 µg/ml) and HPGP (6.5-13.0 µg/ml) were perfused (16-30 µl/min) into the ventral septal area, utilizing push-pull cannulae, in conjunction with intravenous (iv) or intracerebroventricular (icv) administration of the pyrogen (S. abortus equi). When AVP was perfused, fever was reduced whether the pyrogen was administered systemically or centrally. Perfusion of the vehicle or AVP by itself had no measurable effect on resting body temperature. However, when pyrogen was administered during perfusion with the vehicle alone, the typical biphasic pyrogen fever, accompanied by peripheral vasoconstriction, was evoked. Conversely, when HPGP was perfused similarly, an unusual, long latency hyperthermia was elicited. The characteristics of this febrile response, unlike that evoked by pyrogen alone, included marked, whole body shivering but not vasoconstriction.

In contrast to previous reports concerning the action of AVP on thermoregulation and fever in the rabbit, these results suggest that this pituitary neuropeptide has marked antipyretic action in the rabbit. Thus, in accordance with previous findings that have established AVP as an endogenous antipyretic in the sheep, rat, and guinea pig, we concluded this peptide has similar site specific actions in the rabbit.

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326.8 THE MODULATORY ROLE OF PERIPHERAL VASOPRESSIN ON BEHAVIORAL HABITUATION IN THE RAT IS MEDIATED BY SEPTAL REGULATION *OF* CA4-AREA DENTATA SENSITIVITY TO CENTRAL VASOPRESSIN. Cerbone, A.* and Sadile, A.G. Inst.Human Physiol. & Med.Physics, 1st Med.Sch., Univ.Naples, Naples, Italy.

We have previously shown a facilitatory effect of a single peripheral post-trial injection of vasopressin on long-term behavioral habituation (LT-HAB) to a novel environment in the albino rat (Sadile, A.G. et al., Abh.Akad.Wiss.DDR, 5: 203, 1979), at doses which induced neither proactive effects on sensorimotor capacities nor viscero-gustatory conditioning to sweet taste. The steep retrograde hypermnesic gradient raised the possibility of a classical conditioning effect with the novel environment acting as CS and some visceral VP effect as UCS, an hypothesis developed thenafter by Le Moal, M. et al. (Nature, 291: 491, 1981). This facilitatory effect of post-trial VP on LT-HAB turned out to be a biphasic one with inhibition at low (4.4ng.100gm-1, b.wt.) and facilitation at higher doses (44 or 440ng), which suggested a modulatory effect of peripheral VP, at least upon this model of behavioral plasticity. Furthermore, the differential dose-response profile in rats of Naples High (NHE) and Low Excitable (NLE) strains (Sadile, A.G. et al., Neurosci.Lett., S7: 51, 1981), differing in behavioral arousal to spatial novelty (Sadile, A.G. et al., Soc.Neurosci.Abstr., 9: 643, 1983) and the ability of micropressure applied vasopressin to elicit DC slow field depolarization of CA4-area dentata elements (1pg to 10ng), suggested the modulatory effect of VP on behavior to be mediated by tuning of neuronal excitability , through activation of "high affinity"in vivo systems for VP, at least at the entorhino-dentate interface studied (Sadile, A.G. et al,.Behav. Brain Res., 5: 117, 1982). Moreover, subchronic elevation of plasma VP increased the sensitivity of hippocampal CA4-area dentata to VP-induced slow field depolarization, suggesting a positive feed-back between peripheral VP and hippocampal sensitivity to central VP (Sadile, A.G., Soc.Neurosci.Abstr., 8: 367, 1982), which was abolished by electrolytic lesion of medial septal nucleus (MSN)(in preparation). Finally, the inability of central or peripheral V1 vasopressor receptor antagonist n(CH_2)$_5$MeTyr AVP to block the effect of peripheral VP upon LT-HAB to spatial novelty, seems to rule out the involvment of this VP receptor type in long-term behavioral habituation. Our experimental evidence supports the hypothesis of a modulatory role of peripheral VP upon sensitivity of hippocampus to central VP or its fragments (Burbach, J.P.H. et al, Science, 221: 1310, 1983), through the septal input.

326.9 CHARACTERIZATION OF THE THERMOREGULATORY EFFECT OF ARGININE VASOPRESSIN IN THE RAT. W.D. Ruwe, A.M. Naylor* and W.L. Veale. Department of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Arginine vasopressin (AVP) has been shown to have a variety of effects on thermoregulation in the rat including hypothermia and hyperthermia. In addition, in other species AVP has been shown to suppress fever of bacterial origin. The present study was undertaken to more fully characterize the thermoregulatory action of this neuropeptide.

Male, Long Evans rats (280-325 g) were anaesthetized with pentobarbitol and stainless steel guide tubes were implanted above the ventral septal area and the lateral cerebral ventricle (LCV). Bilateral microinjections of 100 ng/0.5 µl AVP into the ventral septal area evoked a marked hyperthermia of more than 1°C within 60 minutes after microinjection. This increase in core temperature was partially antagonized by the prior administration of a vasopressor antagonist d(CH_2)5 D-Tyr V AVP. In confirmation of earlier reports, infusion of 1.0 µg/10 µl of AVP into the LCV evoked a short-lived hypothermia of approximately 1.0°C. Sites of the rostral diencephalon at which Prostaglandin E_2 (PGE_2) evoked a hyperthermic response were identified by microinjecting 100 ng/0.5 µl of this thermogenic agent Subsequent push-pull perfusion of AVP (6.5 µg/ml at 16.0 µl/min) in these sites was effective in attenuating the hyperthermic response to an intracerebroventricular (icv) administration of this putative mediator of fever (2.0 µg/10 µl).

These results indicate that the effects of AVP on the thermoregulatory system are dependent upon the site and route of administration. Moreover, use of the push-pull perfusion technique, which may more closely approximate the in vivo release of neurotransmitters, offers confirmatory evidence that AVP may be involved in the suppression of fever.

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326.10 SEX AND ESTROUS CYCLE DIFFERENCES IN NEUROPEPTIDE CONCENTRATION IN DISCRETE BRAIN REGIONS AND POSTERIOR PITUITARY OF THE RAT. M. Frankfurt, R. A. Siegel*, I. Sim*, and W. Wuttke*, German Primate Center, 3400, Goettingen, FRG.

Morphological and biochemical sex differences have been reported in several rat brain regions (Gorski, et al., J. Comp. Neurol., 193: 529-539, 1979; Luine and McEwen, Neuroendocrinolgy, 36: 475-482, 1983). These are thought to result from neonatal exposure to gonadal steroids. Furthermore, neuronal systems in the adult rat appear to be influenced by gonadal steroids. Therefore,in the present study we have examined sex and estrous cycle differences in cholecystokinin (CCK) and substance P (SP) in discrete brain regions.

Male (M) and female (diestrous, D; estrous, E; proestrous, P) rats were decapitated and the brain, median eminence (ME) and posterior pituitary (PP) removed to liquid nitrogen. Brain areas were microdissected frozen and tissue extracted for neuropeptide radioimmunoassay. Data were analyzed by analysis of variance or Kruskal H-test followed by t-tests.

CCK concentrations were approximately 2 fold higher in M than D females in the diagonal band of Broca, medial preoptic area, ventromedial hypothalamic area, ventral tegmental area, entorhinal and cerebral cortex. No sex differences in SP were observed in these areas. In the lateral septum, amygdala and periventricular grey CCK was lower in P than in D. SP was lower in P than in both E and D in the lateral septum, medial septum, lateral preoptic area and medial preoptic area. In the periventricular grey and ventral tegmental area SP was lower in P than in D.

Significant sex differences in CCK and LH-RH were observed in the ME, while SP and somatostatin were unchanged. In the PP, oxytocin was 2 fold higher in D than in M.

Taken together these results suggest that the neuropeptide transmitters may be involved in sexual differentiation as well as gonadal steroid feedback.

326.11 ARGININE VASOPRESSIN (AVP) ALTERS THE MORPHOLOGY OF THE RAT CHOROID PLEXUS (CP) IN VITRO T. M. Liszczak, P.Mcl. Black, L. Foley,* Neurosurgical Service, Mass.General Hospital & Harvard Medical School, Boston,Mass. 02114. We have previously shown that intraventricular arginine vasopressin (AVP) increases cerebrospinal fluid (CSF) absorption in the rabbit but does not change CSF formation. As the choroid plexus may be involved in both formation and absorption of CSF, the present study was undertaken to evaluate morphological changes suggesting water transport in the choroid plexus exposed to AVP. Choroid plexus was rapidly removed from 67 rats and incubated with 10-8 to 10-12 molar AVP in Hanks balanced salt solution for one to two hours at 24°C. These concentrations correspond to 11,100 to 1.11 pg/ml. There were 3 control groups: animals whose choroid plexus was incubated in AVP diluent, and animals incubated with the same concentrations of oxytocin, and lysine vasopressin. After incubation, tissues were fixed with 2.5 % glutaraldehyde, and postfixed with osmium tetroxide. Some were critical point dried and prepared for scanning electron microscopy, others were embedded in Epon-812, sectioned and examined by transmission electron microscopy. Strips of choroid plexus containing 100 cells were examined in each incubation fluid for evidence of lateral or subcellular fluid accumulation. There was a rounding of choroid plexus epithelial cells and an accumulation of fluid in the lateral and subcellular spaces with incubation in 10-8, 10-9 and 10-12 (1.11 pg/ml) molar AVP. These changes were not seen in choroid plexus incubated with lysine vasopressin, oxytocin, or diluent. These data suggest that arginine vasopressin may cause fluid absorption in the choroid plexus.

326.12 NOREPINEPHRINE-LIKE EFFECTS OF NEUROPEPTIDE Y (NPY) AND HUMAN PANCREATIC POLYPEPTIDE (hPP) ON LH RELEASE. Satya P. Kalra* and William R. Crowley* (SPON: G. Freund) Dept. of Ob-Gyn, Univ. Fla. Col. Med., Gainesville, FL, 32610 and Dept. of Pharmacol Univ. Tenn. Col. Med., Memphis, TN 38163

Recent immunocytochemical mapping studies have revealed overlapping distribution patterns for NPY and LH releasing hormone neurons and also the coexistence of NPY and adrenergic transmitters in neurons in the hypothalamus. Since adrenergic agonists exert profound effects on LH secretion, we have investigated whether NPY and hPP, a polypeptide with considerable amino acid sequence homologies with NPY, may have norepinephrine-like effects on LH release. Ovariectomized rats were implanted with permanent cannulae in the third ventricle of the brain and allowed to recover for two weeks. They were divided into 2 groups: one group was left untreated (Ovx) and the other was primed with estradiol benzoate (EB, 30 µg/rat) and progesterone (P, 15 mg/rat). Two days later, the effects of intraventricular NPY or hPP in saline and saline alone (control) on LH levels were assessed in blood samples withdrawn at 0, 10, 20, 30 and 60 min from intrajugular cannulae. NPY and hPP (0.5 or 2.0 µg) produced marked decreases in plasma LH levels within 10-20 min in Ovx rats. The decrease in LH levels displayed an "all or none" characteristic since the low dose (0.1 µg) was completely ineffective while higher doses of 0.5-2.0 µg attenuated LH release in a similar manner. In contrast, NPY and hPP readily stimulated LH release in EBP-primed Ovx rats. hPP (2 and 10 µg) significantly increased circulating LH levels at 10 min in a dose-related fashion. NPY was more effective than hPP since similar dose-related increments in plasma LH levels were seen at doses of 0.5 to 2.0 µg and thereafter, the response plateaued as 5 times higher concentrations of NPY failed to further augment LH release. These studies show that the ovarian steroid milieu may dictate the direction of NPY or hPP-induced LH response in a manner similar to that observed after intraventricular injection of norepinephrine and they further raise the possibility that NPY may participate in the hypothalamic regulation of LH release either independently or in concert, when co-released, with adrenergic transmitters.

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326.13 ACTION OF OVARIAN HORMONES ON THE SENSITIVITY OF MIDBRAIN CENTRAL GRAY NEURONS TO LHRH. A. Chan*, C.A. Dudley and R.L. Moss (SPON:R. Tindall). Dept. Physiol., UTHSCD, Dallas, TX 75235.

The midbrain central gray (MCG) has been implicated in the expression of mating behavior in female rats. Since microinfusion of luteinizing hormone-releasing hormone (LHRH) into the MCG has been reported to induce mating, and since bioactive LHRH, immunoactive LHRH, and neurons sensitive to LHRH have also been reported to be present in MCG, the endogenous decapeptide could be essential for mediating female sexual behavior. The present experiment was designed to study the influence of ovarian hormones, estrogen (E) and progesterone (P), on the sensitivity of MCG neurons to iontophoretically applied LHRH in urethane anesthetized, ovariectomized (OVX) female rats. Extracellular potentials were recorded via a 4 M NaCl-filled center barrel (3-8 MΩ) of a multi-barrelled micropipette. The outer barrels contained one of the following chemicals: (1) LHRH (1 mM, pH 5-6), (2) dopamine (0.5 M, pH 3-4), and (3) prolactin (0.1 mM, pH 7.6). The results are summarized below:

Treatment	Agent iontophoresed:	Number of MCG neurons tested	Response Profile ↑ n	↑ %	↓ n	↓ %	→ n	→ %
E-P Primed	prolactin	33	6	18	3	9	24	73
OVX rats	dopamine	31	4	13	7	23	20	73
(n=86)	LHRH	32	3	9	13	41	16	50
Untreated	prolactin	35	4	11	1	3	30	86
OVX rats	dopamine	19	0	0	3	16	16	84
(n=61)	LHRH	31	0	0	1	3	30	97

A total of 147 single neurons were recorded and histologically localized in MCG. The mean firing rate of MCG neurons was the same in hormone primed and non-primed animals. Further analysis of the results indicates that there was a significantly higher ($p<0.001$) percentage of MCG neurons demonstrating an inhibitory response to iontophoretically applied LHRH in E-P OVX rats than in untreated OVX rats. Such a difference was not observed with respect to prolactin and dopamine. The above findings provide evidence at the electrophysiological level that ovarian hormones modulate the responsiveness of MCG neurons to LHRH. The modulatory effect was specific in that responsiveness to dopamine and prolactin was unchanged. The results suggest that MCG neurons are more sensitive to LHRH when the hormonal status of the animal is that which occurs during sexual heat.

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326.14 DIFFERENTIAL ACTIONS OF LHRH AND ITS BEHAVIORALLY ACTIVE FRAGMENT ON HYPOTHALAMIC NEURONAL ACTIVITY. C.A. Dudley, M.J. Twery, and R.L. Moss. Dept. Physiol., UTHSCD, Dallas, TX 75235.

A fragment of the LHRH molecule, Ac-LHRH$^{5-10}$, facilitates mating behavior in the female rat while having no effect on LH release. The present study was designed to compare the action of Ac-LHRH$^{5-10}$ and LHRH on single unit activity in the medial basal hypothalamus and to assess the influence of exogenously administered estradiol benzoate (EB) on this action. Single unit activity was recorded through the center barrel (4M NaCl) of a multibarrelled glass micropipette which was lowered through the dorsomedial (DM) and ventromedial (VM) hypothalamus (H) of urethane anesthetized, ovariectomized female rats. Ac-LHRH$^{5-10}$ (1mM) and LHRH (1mM) were iontophoresed through the outer barrels. A total of 115 neurons were tested with the fragment, 101 were tested with LHRH, and 80 were tested with both. The number of neurons excited (↑), inhibited (↓), or non-responsive (→) to the two drugs is tabled below. In non-primed animals, the majority of neurons were not affected by either agent. EB priming resulted in a shift in sensitivity to Ac-LHRH$^{5-10}$, from → to ↓, in both the DMH and VMH. A similar shift in sensitivity, in response to LHRH was observed only in the VMH. In non-primed animals, a small percentage of neurons responded differently to the two agents (eg: Ac-LHRH$^{5-10}$↓; LHRH→). EB priming increased the percentage of VMH, but not

		Ac-LHRH$^{5-10}$ ↑	↓	→	LHRH ↑	↓	→	(n) tested with both, % responding differently	
Non-primed N=13	DMH	3	5	27	0	7	24	(21)	27.2
	VMH	2	8	23	2	8	19	(22)	28.5
EB primed N=12	DMH	1	8	14	2	2	16	(19)	31.5
	VMH	2	11	11	2	14	5	(18)	55.5

DMH, neurons exhibiting differential responses. The results demonstrate that Ac-LHRH$^{5-10}$ can affect hypothalamic neuronal activity, that the neuronal response to the fragment can be different from the response to LHRH, and that the action of both agents is modulated by EB. EB increased differential responding in the VMH but not in the DMH. Studies attempting to isolate neurons involved in LH release from those involved in mating behavior on the basis of projection pathways and responsiveness to the behaviorally active fragment are presently in progress. Supported by NIH NS 10434.

326.15 SUBSTANCE P-LIKE PEPTIDE IN PREGANGLIONIC PARASYMPATHETIC NERVE TERMINALS OF THE BULLFROG. C.W. Bowers, L.Y. Jan and Y.N. Jan. Department of Physiology, UCSF, 94143.

The preganglionic terminals of the parasympathetic ganglia in the bullfrog atrial septum exhibited intense staining for substance P (SP) using routine immunohistochemical methods. RIA of the septum with a different SP antibody indicates ~1pmol of SP-like material per septum. Since nicotinic cholinergic transmission occurs in these neurons during preganglionic stimulation and since all of the terminals on the neurons appear SP-positive, it is very likely that acetylcholine and a SP-like substance occur in the same terminal. EM immunocytochemistry indicated that the SP-like antigen is associated only with the large dense-cored vesicles in terminals and preterminal axons.

Although the immunohistochemistry suggests a transmitter function of a SP-like substance in this system, local application of SP(10^{-4}M) to septal neurons during intracellular recording did not affect membrane potential, membrane resistance, calcium currents or the shape of the somal action potential. Consistent with recent reports of SP increasing the rate of desensitization of nicotinic receptors, SP(10^{-4}M) enhanced the rate of repolarization of septal neurons during prolonged (300-1000ms) carbachol-induced depolarizations. SP did not affect the magnitude of single nerve-evoked EPSPs though the prolonged summation of EPSPs at high frequencies was partially inhibited in a manner similar to the carbachol-induced depolarization. Synaptic responses induced by stimulation of the preganglionic nerve at 5-20 Hz for 10-30 seconds are blocked by cholinergic antagonists in most neurons. Some neurons exhibited small (~1mV) membrane potential changes lasting ~1 min in the presence of cholinergic antagonists, but these effects could not be mimicked by 10^{-4}M SP.

Recent work with HPLC demonstrated that the SP-like antigen in the septum does not coelute with SP, physalaemin, eledoisin or bombesin. However, the septal antigen does behave very similarly to substance K, a recently described substance P-like peptide isolated from spinal cord (Kimura et al., Proc. Jpn. Acad. Ser. B 59, 101, 1983).

The results indicate that a SP-like peptide, possibly substance K, is present in a vesicle population of preganglionic parasympathetic nerve terminals in the bullfrog heart. Intracellular recording techniques indicate that the substance is not producing electrophysiological events commonly associated with synaptic transmission.

326.16 **VASOPRESSIN MAY BE A TRANSMITTER OF SLOW POTENTIALS IN GUINEA PIG INFERIOR MESENTERIC GANGLIA.** S. Peters* and D. L. Kreulen. Department of Pharmacology, University of Arizona, Tucson, Arizona 85724.

Mammalian prevertebral sympathetic ganglia contain a variety of biologically active peptides, one of which is arg^8-vasopressin (AVP). We recently reported that AVP applied by pressure ejection produced a membrane depolarization and an increase in input resistance in a population of inferior mesenteric ganglion (IMG) neurons. The present study continues our investigation into the effects of AVP on IMG neurons and on slow synaptic transmission. IMGs from guinea pigs of either sex were excised and pinned in a recording bath perfused with oxygenated Krebs solution at 37°C. The associated nerves were placed in bipolar platinum electrodes for stimulation. Intracellular recordings were made with glass microelectrodes having tip resistances of 30-70 MΩ. Cholinergic fast excitatory postsynaptic potentials (EPSPs) were elicited by single nerve shocks (0.1 to 0.4 ms), and noncholinergic slow EPSPs by supramaximal repetitive stimulation (20 Hz, 4s). AVP (0.5 to 1.0 μM) superfused over the IMG caused membrane depolarizations ranging from 1.5 to 10.0 mV (5.1±0.5 mV, $\bar{x}$±S.E.M.) in 68% (21 of 32) of cells tested. Depolarizations were accompanied by increases in membrane resistance of 24 to 54% (40±6%) in 4 of 5 cells; one cell showed no change in resistance. The AVP-induced depolarization was not affected by low-Ca (0.25 mM) Krebs. To determine whether AVP mediates slow potentials, control slow EPSPs were compared to those produced in the presence of AVP. Control amplitudes ranged from 2.3 to 8.6 mV (n=9). Subsequent AVP application produced depolarizations in 5 of 9 cells, at the peak of which the membrane potential was manually clamped to the original level and the second slow EPSP was evoked. In 4 of the 5 cells, slow EPSPs produced in the presence of AVP were either partially or completely abolished (73±16% attenuation), while fast EPSP amplitudes were enhanced, probably due to the increased membrane resistance. Slow EPSP amplitudes returned to control levels after 40 min of Krebs washout. Thus, AVP has a direct excitatory effect on IMG neurons. Some slow EPSPs are reduced or abolished in the presence of AVP, suggesting that the transmitter of these slow EPSPs and AVP are acting on the same postsynaptic receptors. These data support a role for AVP as a transmitter of slow potentials in guinea pig IMG. Support HL27781, HL01136.

326.17 BLOOD PRESSURE RESPONSES TO VASOPRESSIN IN VASOPRESSIN-SENSITIZED RATS. D. Lawrence and Q.J. Pittman, Dept. of Pharmacology & Therapeutics, The University of Calgary, 3330 Hospital Dr., N.W., Calgary, Alberta T2N 4N1

The central administration of arginine-vasopressin (AVP) elicits behavioural and convulsive effects. Pretreatment with AVP or AVP releasing stimuli (hemorrhage or hypertonic saline) results in a subsequent supersensitivity to the behavioural and convulsive effects of AVP. In order to examine the mechanism of this supersensitivity, the blood pressure responses to centrally and peripherally administered AVP was tested in control and AVP treated animals. Male Sprague Dawley and Long Evans rats implanted with lateral ventricular cannulae were injected centrally with either 1 μg of AVP or 5 μl of artificial CSF. Twenty-four hours later, under urethane anesthesia, the femoral blood pressure was recorded in control and AVP treated animals. Dose response curves for the central and peripheral pressor effects of AVP were then compared between control and AVP treated animals. The central pressor responses to AVP doses of 0.3, 1 and 3 μg icv in control Long Evans rats were 29 ± 11, 36 and 37 ± 11 mmHg respectively while in treated rats the response to the same doses of AVP were reduced to 15 ± 3, 29 ± 13 and 28 ± 7 mmHg respectively. There was little difference between the peripheral pressor responses to AVP in AVP treated or control animals. These effects were observed in both strains of rat. The results suggest that the mechanism of AVP supersensitivity is not mediated through changes in the AVP receptor itself but is a consequence of an AVP action which is not associated with the central pressor effect of AVP.

Supported by the Canadian MRC; D. Lawrence is an AHFMR Postdoctoral Fellow.

327.1 CONTRIBUTIONS OF THE AUTONOMIC NERVOUS SYSTEM (ANS) TO CARDIOVASCULAR ACTIONS OF γ-MSH IN UNANESTHETIZED RATS. M. F. Callahan, R.F. Kirby, A.K. Johnson, J.R. Lymangrover, and K.A. Gruber* Depts. of Psychology, Pharmacology, and The Cardiovascular Center, University of Iowa, Iowa City, IA.

γ-MSH is a member of the pro-opiocortin peptide class, possessing both natriuretic and hypertensive actions. Other members of this class of peptides produce peripheral SNS activation causing both increased blood pressure(MAP) and heart rate(HR). The studies reported here assess ANS contributions to the CV actions of γ-MSH and the direct adrenergic agonist phenylephrine (PHE).

Male Sprague-Dawley rats were instrumented with catheters in the right common carotid artery for determination of MAP & HR, and jugular vein for drug infusions. Following adaptation to the testing chamber, all animals were given infusions of PHE (3-5 ug/100ul) and γ-MSH (10-20 ug/100ul) designed to produce a 30-70 mmHg increase in MAP. Animals were then subjected to one of four experimental proceedures: I.α_1-adrenergic blockade (prazocin, 0.5mg/kg) II.β_1-adrenergic blockade (metoprolol, 1.0mg/kg) III. cholinergic blockade (methyl atropine, 0.5mg/kg) IV. ganglionic blockade (chlorisondamine, 0.5mg/kg). Then, all animals were tested to PHE and γ-MSH. The following table represents the change from baseline (x ± s.d.) in response to PHE or γ-MSH.

	Before Blockade		After Blockade	
	MAP	HR	MAP	HR
I. α_1-blockade(n=7)				
Δto PHE	61 ± 17	-162 ± 65	11 ± 18	-13 ± 18
Δto MSH	59 ± 9	17 ± 45	13 ± 8	-7 ± 69
II. β_1-blockade(n=6)				
Δto PHE	51 ± 9	-108 ± 21	51 ± 8	-87 ± 24
Δto MSH	55 ± 7	-19 ± 46	40 ± 26	-13 ± 21
III Cholinergic-blockade(n=6)				
Δto PHE	44 ± 15	-100 ± 49	47 ± 10	-18 ± 24
Δto MSH	44 ± 20	32 ± 35	31 ± 12	17 ± 9
IV. Ganglionic-blockade(n=5)				
Δto PHE	66 ± 12	-129 ± 9	81 ± 14	-17 ± 26
Δto MSH	59 ± 11	13 ± 56	10 ± 10	11 ± 17

These data indicate that γ-MSH produces an increase in MAP by a centrally mediated increase in SNS activity and inhibits baroreceptor function. We are currently investigating the hypothesis that γ-MSH inhibits baroreceptor function by influencing vagal efferent activity.

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327.2 SOMATOSTATIN AND B50 PROTEIN PHOSPHATASE. L.A. Dokas, E. Apolonio*, L. Jeziorowski*, D. Ondrejka* and D.H. Coy*. Medical College of Ohio, Toledo, OH 43699 and Tulane University, New Orleans, LA 70112.

Preincubation of rat cortical synaptic plasma membranes (SPM) for 30 min with [D-Trp^8]-somatostatin (D-Trp^8-SS) completely inhibits subsequent phosphorylation from [γ-^{32}P]-ATP of the presynaptic protein B50 (48,000 daltons, IEP of 4.5). In addition, when SPM are prelabeled with [γ-^{32}P]-ATP and then incubated with D-Trp^8-SS during a period of dephosphorylation, the peptide diminishes the loss of label from B50. To determine the enzymatic basis for these effects, B50 protein kinase and an analogous protein phosphatase fraction from rat brain were purified for studies with D-Trp^8-SS. Crude rat brain membranes were treated with 0.5% Triton X-100 - 75 mM KCl and the extract was chromatographed on a DEAE cellulose column using a 0-400 mM NaCl gradient as described by Zwiers et al. (J. Neurochem. 34:1689). Consistent with these earlier studies, B50 and B50 protein kinase co-elute at about 200 mM NaCl. The major portion of protein phosphatase activity, assayed with ^{32}P-labeled B50 as a substrate, separates from B50 - B50 protein kinase at 260 mM NaCl. When D-Trp^8-SS is added to the column-purified protein phosphatase, it inhibits the dephosphorylation of B50.

Subcutaneous injection of 300 mg/kg of cysteamine (2-mercaptoethylamine) into rats for 24 hours decreases the somatostatin-like immunoreactivity of the cortex by -56.0 ±7.5% (N=3), compared to values from control rats. Concommitant with this action, an increase in the in vitro phosphorylation of B50 is seen in cortical SPM from cysteamine-treated rats. No change is seen in the phosphorylation of any cytosolic protein after cysteamine administration. The effect of cysteamine on B50 phosphorylation is consistent with less in vivo inhibition of protein phosphatase in synaptic membranes due to diminished levels of endogenous somatostatin. Both in vivo and in vitro approaches, therefore, indicate one action of somatostatin in the brain is the inhibition of B50 protein phosphatase. Supported by grants AG 04190 and BRS 5 S01 RR 05700 13 to L.A.D. and grant AM 18370 to D.H.C. Drs. Henk Zwiers and Willem H. Gispen of the University of Utrecht, The Netherlands, are acknowledged for their collaboration in the purification of B50 protein kinase and phosphatase fractions.

327.3 THE EFFECTS OF IONOPHORETICALLY APPLIED SUBSTANCE K (SK) ON SINGLE UNIT ACTIVITY IN THE RAT SUBSTANTIA NIGRA (SN). T.H.Lanthorn, T.L. O'Donohue, C.W.Shults, T.N.Chase, J.R.Walters. NINCDS, Bethesda, MD 20205.

Two substance P (SP) precursors were recently identified.[1] One precursor contains only SP and the other contains SP and SK. A large SP-like projection from the striatum to the SN has been shown, but ionophoretically applied SP has been reported to have weak and inconsistent excitatory effects on single cell activity in the SN. Recent results have shown that SK is contained in the SN in similar concentrations to SP.[2] The present study was undertaken to determine if SK is an active compound in the SN and to complement ongoing anatomical investigations of SK.

SN extracellular action potentials were recorded in chloral hydrate-anesthetized rats with 5-barrel micropipettes. The 3 drug barrels contained SK (0.5-1mM in water or 100mM NaCl,pH5), SP (1mM in 100mM NaCl,pH5), L-glutamic acid (GLU;100mM,pH7), GABA (1mM in 150mM NaCl,pH4) or 100mM NaCl. Release of SK was confirmed by RIA. In some cases SK was ejected by pressure.

SK was applied to 8 dopamine-type (DA) cells. With ejection currents up to 40nA (27±4) SK had no significant effect on these cells (<20% baseline). SP had no effect on 2 DA cells. On other SN cells, SK produced different effects. On 17 cells SK (up to 105nA;47±8) had no effect on firing rate nor on effects of GLU (n=5) or GABA (n=2). On 5 of these cells SP also had no effect. On 3 cells SK had a strong inhibitory effect (complete inhibition at 1-15 nA). On 9 cells SK was weakly inhibitory (20-40% at 15-50nA). SP was tested on 3 of these cells and had weak, variable effects. On 12 cells SK had a strong excitatory effect (35-300% increases over basal firing rate at 8 -25nA). This excitation began 5-25 sec after ejection current was applied and lasted 20-60sec after ejection current was removed. This excitatory effect was mimicked by SP although SP had a longer latency (>30sec). The cells which were excited by SK could be differentiated anatomically. Almost invariably these cells were recorded in very close proximity to a DA-type cell; histologically they were all located in or immediately adjacent to the pars compacta. The distribution of SK binding in the SN is largely confined to the dorsal SN.[2]

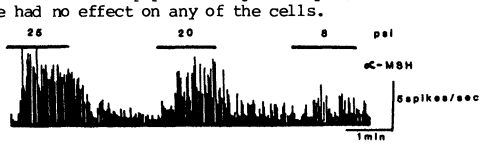
The results suggest that ionophoresis of SK may inhibit some cells in the pars reticulata, although conditions for this inhibition are unclear. In addition, SK, and SP as well, can powerfully excite a population of cells in or near the pars compacta of the SN. These cells are not DA cells as they are usually defined but are found in close proximity to DA cells.

[1]Nawa et al., Nature 306:32, 1983
[2]Shults et al., Soc. for Neurosci., 1984

327.4 AN IN VIVO STUDY OF THE EFFECTS OF α-MSH ON THE FIRING RATE OF SINGLE NEURONS IN THE PARAVENTRICULAR NUCLEUS (PVN) OF THE RAT THALAMUS. C.M. Haws and G.R. Siggins. (SPON: J. Aldenhoff). Div. Preclin. Neurosci and Endocrin., Scripps Clinic and Research Foundation, La Jolla, CA.

α-melanocyte-stimulating hormone (α-MSH) is 13 amino acid peptide derived from a prohormone precursor, pro-opiomelanocortin (POMC; Mains et al., J. Biol. Chem. 251, 4115, 1976). Immunohistochemical studies demonstrate a high density of POMC-positive cell bodies in the region of the mediobasal hypothalamus with projections to various regions of the brain, including the PVN of the thalamus (O'Donohue et al., Neurosci. Lett. 14, 271, 1979). The potent behavioral actions observed following microinjection of α-MSH and related peptides into localized areas of the thalamus suggest a direct action of this peptide on the CNS. The present study was aimed at observing the effects of α-MSH on the firing rate (FR) of single neurons in the PVN.

α-MSH (1 mM in Hepes buffer, pH 7.3) was applied by pressure ejection (6-30 psi) from 5-barrel micropipettes onto glutamate-driven neurons in the thalamus of chloral hydrate anesthetized rats. The position of the recording electrode was marked by iontophoresing Pontamine Sky Blue (saturated solution in 3M NaCl) and later verified histologically. Of 37 neurones studied, 5 cells (13%) showed a marked "dose-related" excitation (see Figure) which was characterized by a rapid onset and offset (10-20 sec), 4 cells (10%) showed a weaker but long-lasting inhibition of FR that exceeded the duration of application by up to 240 sec. The remaining cells (77%) exhibited no response to the application of the peptide at up to 30 psi, and the vehicle alone had no effect on any of the cells.



These results thus demonstrate that α-MSH has a direct action on the FR of a sub-population of neurons in the PVN of the thalamus. This suggests a possible role for this peptide in the CNS as a neurotransmitter or co-transmitter released with other POMC peptides. Possible interactions of α-MSH with these other peptides are currently being investigated. (Supported by the MRC and the USPHS DA 03665.)

327.5 ACTIONS OF CHOLECYSTOKININ OCTAPEPTIDE ON RAT SPINAL DORSAL HORN NEURONS IN VITRO. J. Willetts,* L. Urban,* K. Murase and M. Randic (SPON: D.G. Emery). Dept. of Vet. Physiology and Pharmacology, Iowa State University, Ames, IA, 50011.

Membrane actions of cholecystokinin octapeptide (CCK-8) and effects on the Ca-dependent action potential of dorsal horn neurons have been investigated using intracellular recording techniques in the rat (10-25 days old) spinal cord slice preparation. Bath application of CCK-8 (5×10^{-10} to 10^{-5} M) caused a reversible, dose-dependent depolarization and an increase in excitability (n=57) in about half of the cells examined. Depolarization was almost regularly accompanied by an increase in synaptic activity and occasionally firing of action potentials. CCK-8 increased input resistance in 5 of 9 tested cells, and decreased resistance in 3 cells. While CCK-8-induced depolarization was present in slices obtained from capsaicin-treated rats (50 mg/kg, s.c. on day 2), proglumide (DL-4-benzamido-N, N-dipropylglutaramic acid, 10^{-6} to 10^{-5} M) reduced or abolished the response. After blocking synaptic activity by perfusion of the slices with TTX (10^{-6} M) or low Ca, high Mg solution, CCK-8 still evoked a depolarizing response, although its amplitude and duration was reduced. In a medium containing both TTX (10^{-6} M) and TEA (2×10^{-2} M), CCK-depolarization was present and firing of Ca spikes elicited. In addition, CCK-8 produced a reversible decrease in Ca spike duration. These data are consistent with a possibility that CCK-8 may have a physiological role in processes underlying sensory information transfer and integration in the spinal dorsal horn.

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327.6 THYROTROPIN-RELEASING HORMONE (TRH) AND NALOXONE IN HEMORRHAGIC SHOCK: EFFECTS ON REGIONAL BLOOD FLOW. Sivam, S.P., Faden, A.I., and Feuerstein, G.; Neurobiology Research Unit, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

TRH and naloxone have been shown to enhance cardiovascular recovery in various experimental shock models, including hemorrhagic shock. One of the important factors in the recuperation in hypovolemic shock is the redistribution of blood to vital organs. This study aimed at elucidating the role of blood flow to discrete organs in hemorrhagic shock and its modification by TRH and naloxone. Studies were conducted in male Sprague-Dawley rats under urethane anesthesia. The blood flow (as mean flow velocity) through the abdominal aorta (AA), superior-mesenteric (MS) and renal (RN) arteries was monitored by the directional pulsed Doppler flowmeter (University of Iowa Bioengineering Resource Facility). Mean arterial pressure (MAP) and heart rate (HR) were also recorded continuously and printed at 1 min intervals on a Northstar Hazeltine computer. Hemorrhagic shock was induced by bleeding via a carotid artery at the rate of 3 ml/300 g body weight/6 min. This protocol of bleeding induced a reduction in MAP (-60 mmHg) and HR (-175 beats/min) at the end of the bleeding; the blood flow to AA, MS and RN regions was reduced (60 - 80% of control) with a concommitant increase in resistance. TRH (2 mg/kg, i.a.) or naloxone (5 mg/kg, i.a.) were injected at the end of bleeding, in 0.5 ml saline. Control group received an equal amount (0.5 ml) of saline which did not alter the cardiovascular derangements which occurred as a result of hemorrhage. Administration of TRH significantly increased the MAP and HR which was accompanied by an increase in blood flow to the discrete organs studied. These effects of TRH were maximal at 5 min (percent change of blood flow in control vs. TRH-treated groups: AA, -52 ± 7 vs. -21 ± 2.1; MS, -47 ± 5 vs. -18 ± 4.3; RN, -78 ± 8 vs. -7 ± 6) and was still significant at 10 min. There was a significant decrease in resistance with the increase in blood flow in the MS and RN arteries. Naloxone in the above model of hemorrhagic shock produced an increase in MAP without eliciting alterations in other parameters except for a trend to increase mesenteric flow. The results suggest that the beneficial effects of TRH and naloxone in hemorrhagic shock may involve different mechanisms.

327.7 INTERMEDIATE ROLE OF BRAIN NEUROTRANSMITTERS ON NEUROTENSIN-INDUCED CYTOPROTECTION. D.E. Hernandez and A.J. Prange, Jr., Biological Sciences Research Center, Dept. of Psychiatry, Univ. of North Carolina School of Medicine, Chapel Hill, N.C. 27514

We have reported previously that intracisternal (IC) administration of neurotensin (NT) produces a dose- and time-dependent reduction of cold-restraint stress (CRS)-induced gastric ulcers in rats without affecting gastric acid secretion (Amer. J. Physiol., G342-G346, 1982; J. Neurosci. Res. 9:145-157, 1983). This effect of NT appears to be mediated by the central nervous system because peripheral (i.v.) NT is totally ineffective in this paradigm. The present study sought to clarify the central mechanism of NT's cytoprotective effect by utilizing pharmacological treatments which alter the function of selected neurotransmitter systems.

Male S-D rats (180-220 g) were food deprived 24 hrs prior to experimentation and then pretreated intracerebroventricularly (i.c.v.) with the following drugs (followed by the dose, route of administration and pretreatment interval): carbachol (2 µg, i.c.v., 30 min), atropine sulphate (25 µg, i.c.v., 30 min), muscimol (50 ng, i.c.v., 30 min), bicuculline (1 µg, i.c.v., 30 min), naloxone (2 mg/kg, I.P., 60 min), methysergide (1 µg, i.c.v., 30 min), cyproheptadine (1 µg, i.c.v., 30 min), haloperidol (5 µg, i.c.v. in 0.3% tartaric acid, 30 min), and methylphenidate (80 µg, i.c.v., 60 min). After this, rats were treated with IC NT (30 µg) or vehicle (10 µl of 0.9% NaCl) and subjected to CRS for 3 hr after which they were killed by decapitation and the stomachs examined for gastric ulcerations.

Pretreatment with i.c.v. agonists and antagonists of Ach, GABA, or 5-HT receptors, or with I.P. naloxone did not significantly alter NT-induced cytoprotection. However, i.c.v. haloperidol (5 µg) totally blocked NT's cytoprotective effect, and methylphenidate (80 µg) produced cytoprotection similar to IC NT.

The present results indicate that NT-induced cytoprotection is not mediated by 5-HT, GABA, Ach receptors or endogenous opiate systems but suggest that this effect of NT may be expressed through interactions with brain DA systems.

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327.8 ANTIPYRETIC ACTIVITY OF A POTENT α-MSH ANALOG. J.M. Lipton and M. Holdeman* (SPON: W.Clark).

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[Nle^4, D-Phe^7]-α-MSH has exceptional potency in certain biological assays of α-MSH activity such as skin darkening in frogs; however, there is no evidence of similar effects involving activity within the CNS. We determined the antipyretic and hypothermic potency of this analog in the rabbit relative to α-MSH. 40 and 80 ng of the analog caused hypothermia when injected into a lateral cerebral ventricle of afebrile rabbits, whereas 20 and 10 ng, which had no effect on the temperature of afebrile animals, effectively reduced fever induced by iv administration of leukocytic pyrogen. These responses indicate that the analog has approximately 10 times the potency of α-MSH. In contrast, iv administration of 16 µg of the analog, an extremely large dose relative to established antipyretic doses of α-MSH, elicited weak and variable responses. Since this analog is said to be resistant to enzymatic degradation by serum enzymes (Sawyer et al., Proc. Natl. Acad. Sci., 77: 5754, 1980), the contrast between the effects of central and peripheral administration may reflect a limited ability of the analog to reach central receptor sites when given iv. The marked central potency of [Nle^4, D-Phe^7]-α-MSH could result from increased duration of action and/or greater affinity for central receptor sites relative to α-MSH. The latter possibility appears more likely since the duration of the hypothermic and antipyretic response elicited by the analog did not significantly exceed those elicited by equipotent doses of α-MSH. (Supported by NINCDS Grant 10046)

327.9 CYCLO (HIS-PRO) (cHP) REGULATES STRIATAL DOPAMINERGIC FUNCTION. J.S. PETERSON*, P.W. KALIVAS*, AND C. PRASAD (SPON:N.W. Pedigo). Depts. of Medicine, Biochemistry, Pathology, and Pharamocology, Louisiana State University Medical Center, New Orleans, La. 70112.

cHP is a cyclic dipeptide derived from thyrotropin-releasing hormone (pGlu-His-ProNH$_2$, TRH) by its limited proteolysis (Prasad and Peterkofsky, J.Bio.Chem.251:3229, 1976). This peptide has been shown to elicit a variety of biologic activities in rodents and primates, as well as to exist in peripheral and CNS tissues. To understand the mechanism(s) of actions of cHP in CNS, we have investigated the cHP effects on striatal dopamine (DA) metabolism.

Subcutaneous injections of cHP (0.5 mg/Kg) to rats led to a time-dependant depletion of striatal DA. Thirty min following treatment, DA levels declined to a nadir of 114.7 ± 14.2 pmols/mg protein (n=9) and then steadily rose to control (225 ± 31 pmols/mg protein, n=5) levels by two hours. Subsequently, DA levels were measured 30 min post-cHP injections. Depletion of DA increased with increasing cHP doses reaching to a max at 0.5 mg/Kg. Since cHP did not alter the striatal HVA and DOPAC, the decreased DA levels could not be due to elevated DA release or metabolism. The effect of cHP on tyrosine hydroxylase (TH), was evaluated by measuring the rate of DOPA accumulation in striatum. Accumulation of DOPA in dopa-decarboxylase inhibitor-treated rats decreased in proportion with cHP doses, reaching to a max at 0.5 mg/Kg, suggesting TH as the possible site of cHP action. However, cHP did not alter TH activity in vitro. The DA depletory effect of cHP was highly specific since TRH, acid TRH or several cHP analogues had no effect.

In conclusion, our data suggest that cHP depletes the striatal DA by inhibiting hydroxylation of tyrosine. Although cHP is a not a direct inhibitor of TH, it may act by modulating the number and/or the kinetic properties of TH molecules.

327.10 SARALASIN INCREASES ACTIVITY OF HIPPOCAMPAL NEURONS INHIBITED BY ANGIOTENSIN II. R.A. Palovcik* and M.I. Phillips. Dept. of Physiol., Univ. of Florida, Gainesville, FL. 32610.

Angiotensin II has been shown to specifically excite neurons in several areas of the rat brain including the hippocampus. In the present study we have found a smaller proportion of neurons which were inhibited by Ang II. In earlier studies where it was found that the Ang II antagonist saralasin inhibited Ang II excitation, saralasin alone decreased spontaneous activity of brain cells. It was hypothesized that the spontaneous activity which was inhibited resulted from stimulation by endogenous brain Ang II (Phillips et al., 1977). However, it was not established whether the inhibition was specific for Ang II or a generalized depressant effect of saralasin. By applying saralasin to those cells in the hippocampus which are inhibited by Ang II, we have tested for excitatory effects of saralasin.

The hippocampus was removed from male Sprague-Dawley rats (150-400g) and sectioned transversely into 0.4mm thick slices. These were maintained in a chamber at 34°C with 95% O_2, 5% CO_2 under continuous perfusion (30 ml/hr) of Yamamoto's solution. Robust spontaneous activity could be recorded as long as 35 hours; however, all experiments were completed by 15 hours post setup. Ang II and (Sar^1,ala^8) saralasin were applied to the perfusion medium by slow injection to avoid mechanical artifacts.

The spontaneous firing rates of neurons tested varied between 0-43 spikes/sec. Of 60 cells, 36 had spontaneous firing rates of 5.5 spikes/sec or less. Thirty-five neurons (of 60) were excited by Ang II and four were inhibited. Some neurons were excited by doses of Ang II as low as 10^{-16} M. Other neurons either showed combined excitation and inhibition or no response. Cells responded throughout the dose range in a dose-response fashion. Saralasin excited cells that were inhibited by Ang II and vice versa.

This implies that the effects of Ang II on hippocampal neurons are specific and secondly, that the decrease in spontaneous activity seen with saralasin is most likely due to an inhibition of endogenous brain Ang II. Since intracellular recordings showed responses to 10^{-16} to 10^{-6} M Ang II, the results indicate that endogenous Ang II has a physiological action in the hippocampus.

(Supported by NSF grant BNS 8025969 to MIP and NIA Fellowship 1F32HL06709-01 to RAP.)

327.11 STRESS INCREASES DOPAC, SUBSTANCE P, AND SUBSTANCE K IN THE A10 BUT NOT A9 REGIONS. J.E. Maggio, A.Y. Deutch, M.J. Bannon, S.-Y. Tam, N. Zamir* and R.H. Roth. Yale University School of Medicine, New Haven, CT and NIMH, Bethesda, MD.

Stress specifically increases the dopamine (DA) metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the prefrontal cortical (PFC) but not olfactory tubercle (TUO) or striatal dopaminergic projections. Immunoneutralization of substance P (SP) in the ventral tegmental area (VTA), source of the cortical DA innervation, blocks stress-induced DOPAC increases in the PFC. We have therefore examined the effect of stress on both SP and DOPAC in the midbrain DA cell group areas, as well as in the striatum and habenula (sources of substantia nigra (SN) and VTA SP, respectively). In addition, substance K (SK), a tachykinin structurally related to SP and derived from a common prohormone, was measured.

Adult male rats were subjected to mild (0.2 mA) footshock stress for 20 min, and sacrificed immediately or 30 minutes later. The VTA, SN, PFC, striatum, and habenula were dissected, and tissue levels of DA, DOPAC, SP, and SK determined. Stress significantly increased DOPAC levels in the PFC, both immediately and 30 min after stress exposure. No changes in either SN or striatal SP levels were observed. In contrast, habenula levels of SP declined while VTA levels increased in response to stress; these changes were accentuated in animals sacrificed 30 min after stress. DOPAC levels in the SN and striatum did not change as a function of stress exposure. However, VTA levels of the metabolite changed in parallel to the observed increases in PFC DOPAC levels. These data indicate that stress results in a selective activation of the SP input to the VTA, but not to the SN. Changes in the presumed sources of midbrain SP reflect alterations in the terminal fields: habenula but not striatal SP concentrations are decreased. Furthermore, the observed changes in SP levels are paralled by a selective rise in VTA (but not SN) DOPAC levels. Preliminary data indicate that changes in regional SK levels follow those changes observed in SP levels.

A10 DA neurons project to both mesolimbic (TUO) and mesocortical (PFC) sites. Stress selectively activates only the PFC DA terminal field. The stress-induced increase in DOPAC in the VTA suggests that a large proportion of the DA neurons in the VTA are activated. However, the DA neurons which project to the PFC represent a relatively small subset of the A10 cell group. These mesocortical DA neurons may therefore exhibit regulatory features which differ from those of other DA cells. (Supported in part by MH-14092).

327.12 EFFECT OF CONTINUOUS ICV INFUSION OF CHOLECYSTOKININ (CCK-8S) ON SLEEP AND RESPIRATION IN THE RAT. S. DeMesquita, W.H. Haney*. Department of Physiology, Marshall University School of Medicine, Huntington, WV 25704-2901.

CCK-8S has been implicated as a central nervous system neuromodulator/transmitter and has been identified in brain stem areas thought to regulate sleep and the automatic control of respiration. Thus a change in the level of brain CCK would be expected to alter control of sleep and respiration. This study examined the effect of chronic elevation of CCK-8S in the cerebrospinal fluid (CSF) on sleep pattern and respiratory rate.

Eight adult male Sprague-Dawley rats, 418 ± 8 g (mean ± SE), were implanted with chronic EEG and EMG electrodes in order to monitor the level of consciousness. A cannula, connected to an Alzet mini-osmotic pump, was inserted into the right lateral cerebral ventricle. Four rats were infused with saline; the remaining rats were infused with CCK-8S, (dose: 1.1 ng CCK-8S/min for five days). The rats were monitored electrophysiologically for four hours each on days 4 and 5 following surgery. A chest balloon was used to record respiratory rate.

Each record was scored into Wake, Rapid Eye Movement sleep (REM) or non-REM sleep (NREM) sections and tabulated. Respiratory rate was analyzed for eight entire REM periods and the preceding five minutes of NREM sleep as well as five minutes of quiet wake for each rat. Breathing rate

RESPIRATORY RATE (breaths/min, mean ± SE)

GROUP	WAKE	NREM	REM
Saline	108 ± 14	86 ± 7	104 ± 3
CCK-8S	76 ± 12	66 ± 5	87 ± 7
Significance	NS	P<0.05	P<0.05

slowed significantly during sleep with CCK-8S. The number of REM periods occurring per hour of NREM sleep was increased (P<0.05) from 3.1 ± 0.8 with saline to 6.1 ± 1.0 with CCK infusion. REM percent was not increased with CCK since the more numerous REM periods tended to be shorter than those with saline infusion.

Chronic infusion of CCK-8S into the CSF significantly depressed respiratory rate during sleep and increased the number of REM periods per hour of NREM sleep. The results suggest a potential physiologic role for CCK-8S in the brain in the regulation of respiratory rate as well as the initiation of the REM sleep phase.

327.13 CCK-8 ELICITS CONTRACTION OF ISOLATED RAT PYLORUS. Randall B. Murphy, James Gibbs, and Gerard P. Smith. New York University, Department of Chemistry, New York, NY 10003 and Cornell University Medical College, Department of Psychiatry, The New York Hospital, Westchester Division, Edward W. Bourne Behavioral Research Laboratory, White Plains, NY 10605.

Binding sites for CCK in the pylorus of the rat have been demonstrated autoradiographically (G.T. Smith et al, Am. J. Physiol. 246:127, 1984). It was suggested that this tissue may have an important role in the ability of exogenously administered CCK-8 to invoke the constellation of behavioral effects which resemble natural satiety. To test this possibility, we have examined the contractile response of the isolated rat pylorus to various pharmacological agents in vitro. The excised tissue was maintained at 35°C in oxygenated Ringer's solution containing 1 mM pyruvate, and 1 µM atropine, and displacement was recorded isometrically. We found that CCK-8 ($5x10^{-10}$ to $3x10^{-7}$M) increased the duration of contraction with an approximate log-linear dose-response function. Although low doses of CCK-8 also increased the frequency and amplitude of contractions, these measures did not correlate with the range of doses of CCK-8 tested. Since CCK-8 acted in the presence of atropine ($1x10^{-6}$M), this suggests that muscarinic cholinergic mechanisms in the pylorus were not necessary for the contractile effect of CCK-8. Preliminary pharmacological analysis suggests that serotonergic and catecholaminergic mechanisms are also not necessary because serotonin, apormorphine, pergolide, papaverine, sulpiride or timolol did not appear to modify substantially the contractile effect of CCK-8. These data indicate that the isolated rat pylorus may have considerable utility as an in vitro assay system for analyzing the effects of analogues and antagonists of CCK.

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327.14 CHRONIC LATERAL HYPOTHALAMIC LESIONS ATTENUATE THE INCREASE IN SERUM GASTRIN INDUCED BY INTRACISTERNAL BOMBESIN. C.V. Grijalva, Y. Taché, M. W. Gunion, J. H. Walsh* and P. H. Cooper. Psychology Department, Brain Research Institute and Center for Ulcer Research and Education, UCLA, Los Angeles, CA 90024

Intracisternal (IC) injection of bombesin in rats reliably increases serum gastrin levels. In a recent study we reported that the increases in serum gastrin levels induced by bombesin were attenuated following acute lesions of the lateral hypothalamus (LH) or transections placed lateral or posterior to the LH (Taché et al., Neuroendocrinology, in press). The present study examined the chronic effects of bilateral electrolytic LH lesions or transections placed anterior, posterior or lateral to the LH in rats on changes in gastric secretory functions and plasma gastrin following IC administration of bombesin.

Three weeks postoperatively groups of male rats were food deprived for 24 h, anesthetized with methohexital (50 mg/kg, IP), and then immediately injected (IC) with either bombesin (500 ng) or an equal volume of saline (10 µl). Two hours later the animals, all conscious, were decapitated and trunk blood and gastric contents collected. The volume of gastric secretion following saline administration was significantly lower in rats with LH lesions or in nonlesioned body weight-matched controls (BWC) [means (ml/2h), LH = 2.9, BWC = 1.5, Normal = 4.3], which were both approximately at 60% normal body weight. However, values for gastric pH, acid output, and serum gastrin did not differ between groups. Bombesin reliably decreased gastric secretory volume and acid output and increased gastric pH in all groups which did not differ significantly on these measures. Gastrin levels also were significantly elevated in all groups following bombesin however, the LH group differed significantly from the Normal and BWC groups [means ± SEM (pg/ml), LH = 201±33, BWC = 317±35, Normal = 385±48], indicating that the differences could not be accounted for by body weight.

These results are in agreement with those from our acute studies (Taché et al., in press) which suggest that the LH area in the rat is involved in bombesin-induced alterations in serum gastrin but not gastric secretory functions.

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327.15 DOES MOSSY FIBRE ACTIVITY RELEASE AN ENDOGENOUS OPIATE IN THE CA2/3 PYRAMIDAL LAYER? T. Dalkara* and K. Krnjevic, Depts. of Anaesthesia Research & Physiology, McGill University, 3655 Drummond St., Montreal, Quebec H3G 1Y6.

Although it is well-known that hippocampal mossy fibres are rich in opioid peptide, and that opiates have a powerful excitatory action in the hippocampus, evidence is lacking that opiate release by mossy fibres significantly affects CA2/3 electrical activity. Following up on the initial studies of Routtenberg et al. (1984, Fed. Proc. 43, 924), we have applied the opiate antagonist naloxone iontophoretically in the CA2 and 3 regions of rats under urethane or Dial anaesthesia while recording extracellularly responses evoked by stimulation, particularly of granule cells/mossy fibres.

Satisfactory placement of the stimulating and recording electrodes was judged by the stereotaxic coordinates, the characteristic field response, and subsequent histological identification of electrode tracks, as well as a Pontamine Sky Blue mark at the principal site of recording.

Three types of responses were examined especially: 1) short latency spikes and PSP fields evoked by single shocks; 2) the striking facilitation of the spike response evoked by a second stimulus at intervals >50-150 ms (depending on the anaesthetic); and 3) the pronounced post-ictal depression that follows stimulation by brief trains at 10 Hz.

Even very prolonged applications of naloxone (from 10-50 mM solutions of naloxone HCl (Sigma) - in 0.15 M saline, at pH 6.5) - in doses as high as 70 nA for 20-30 min - failed to block consistently either the direct population spike response or pair-pulse facilitation, though the powerful excitatory effect of an opiate agonist (FK 33 - 824CH, Sandoz), released from the same electrode, was rapidly abolished by as little as 14 nA of naloxone. We conclude that the principal rapidly-acting excitatory transmitter released by mossy fibres is probably not an opiate.

On the other hand, in a substantial proportion of cases long applications of naloxone greatly reduced or even abolished paired-pulse facilitation. Moreover, in a smaller number of tests, it consistently reduced both the intensity and the duration of post-ictal depression. These observations suggest that an opiate released by mossy fibres can have a significant modulatory action, perhaps by altering the effectiveness of inhibitory tone (Zieglgansberger et al. 1979, Science 205, 415). In addition, it may be released more predictably during seizure activity, and thus reinforce the paroxysmal discharges and the following phase of depression. Supported by the Canadian Medical Research Council.

327.16 SUBSTANCE P (SP) INDUCED EXCITATION OF TRACHEOPULMONARY RECEPTORS AND CENTRAL CHEMOSENSITIVE STRUCTURES. N.R. Prabhakar,* M. Runold,*[1] Y. Yamamoto,*[1] C. von Euler,*[1] J. Mitra and N.S. Cherniack* (SPON: P.E. Crago). Dept. of Medicine, Case Western Reserve Univ., Cleveland, OH 44106; and [1]Nobel Institute for Neurophysiology, Karolinska Institute, Stockholm, Sweden.

Substance P (SP) immunoreactive sensory fibres are present in the tracheopulmonary system (Lundberg et al., Acta Physiol. Scand. 119:243-252, 1983) and in the ventral medullary surface (VMS), in the region of the central chemosensitive structures (Dermietzel et al., Central Neurone Environment, Springer Verlag, 251-256, 1983). We studied the respiratory responses with exogenous application of SP. In anesthetized, spontaneous breathing rabbits (n = 14), right atrial (RA) injection of SP (20 ng to 100 ng/Kg) elicited an "augmented breath" (AB) within 2-3 seconds after the injection. Following AB, respiratory rate and tidal phrenic amplitude increased. Similar injection of SP in the aorta never elicited AB, however, some increase of phrenic activity was present. After vagotomy, AB's observed with RA were absent. RA administration of an SP analog antagonist abolished AB's induced by tracheal occlusion. Recordings from sensory vagal fibers showed that both "irritant" and PDG sensitive "C" fiber activity increased (4.8 ± 0.2 to 9.0 ± 0.8 imp/s irritant receptors, n = 16; 1.6 ± 0.2 to 4.1 ± 0.7 imp/s PDG sensitive "C" fibers, n = 6). In anesthetized, paralyzed, vagotomized cats (n = 6) local application of SP on the VMS (gel foam soaked in 100 µg/ml or 1 mg/ml of SP) increased the efferent activity of phrenic, hypoglossal and recurrent laryngeal nerve activities within 5 to 10 s after application. Hypercapnic response (3% CO_2 in O_2) was increased in presence of SP on VMS. These results suggest that SP may be of physiological importance in the excitation of tracheopulmonary sensory receptors and also the central chemosensitive structures. (Supported by NIH HL-25830; Swedish Medical Research Council 14X00544 and 19X5324)

327.17 BENEFICIAL ACTION OF TRH IN ANAPHYLACTIC SHOCK: EFFECTS OF ACID-TRH AND HISTIDIL-PROLINE DIKETOPIPERAZINE. M. Harel*, J.W. Holaday[1] and S. Amir (SPON: R. Simantov). Dept. Isotope Res., Weizmann Institute of Sci. Rehovot, Israel, and [1]Neuropharm. Br. Dept. Med. Neurosciences, Div. Neuropsychiat., Walter Reed Army Inst. Res. Washington, D.C. 20307.

Thyrotropin-releasing hormone (TRH, PGlu-His-Pro NH_2) has been shown to improve survival in anaphylactic shock in mice. In these studies, TRH appeared to exert its protective effect by acting through the central nervous system (CNS) since survival from anaphylaxis was improved by intracerebroventricular (ICV) administration of TRH at doses that had no effect when given systemically. However, since TRH has been shown to be degraded in the brain to biologically active metabolites, including acid-TRH (TRH-OH, pGlu-His-Pro) and histidil-proline diketopiperazine (DKP, cyclo His-Pro), the specific part of the TRH molecule involved in the central protective action remains to be elucidated. To study the effect of TRH and its metabolites, TRH-OH and DKP in anaphylactic shock, ICR mice were immunized with bovine serum albumin (BSA). Ten days later they were challenged intravenously with BSA (25 ug) followed one min later by an ICV microinjection of saline (control) or saline containing 2.5 or 10 ug peptide. The mortality rate was determined 60 min later. Intravenous challenge with 25 ug BSA produced fatal shock in 100% of the immunized control mice (n=10). ICV TRH significantly improved survival; the mortality rate of shocked mice treated with 2.5 or 10 ug TRH ICV was 40% (n=20) and 20% (n=10), respectively. Similarly, ICV TRH-OH significantly improved survival; the mortality rate following 2.5 or 10 ug TRH-OH ICV was 20% (n=10) and 0% (n=10) respectively. In contrast, ICV injection of 2.5 or 10 ug DKP had no effect on survival from anaphylactic shock. These results indicate that the beneficial effect of centrally administered TRH in anaphylaxis depends on interaction of the molecule or its deamidated metabolite, TRH-OH, with TRH recognition sites in the CNS. The results further suggest that the preservation of the basic TRH structure (i.e. pGlu-His-Pro) is a prerequisite for the central beneficial effect in anaphylaxis. TRH metabolites lacking this structure such as DkP, although biologically active in many test systems, may be ineffective in initiating the central autonomic effects mediating the beneficial action of TRH in anaphylactic shock.

NEUROMODULATORS II

328.1 DETERMINATION OF BRAIN CALMODULIN LEVELS BY HPLC. C.C. Loullis, L. Antonian*, C.E. Rauh*, J. Coupet and A.S. Lippa* Dept. of CNS Res., Medical Research Division of American Cyanamid, Lederle Labs, Pearl River, NY 10965.

The effects of calcium in cellular processes are often mediated by calmodulin (CaM) a small, globular, acidic and thermostable calcium binding protein. In the brain CaM is present at neuronal synapses and has been shown to be involved in neurotransmitter release and receptor sensitivity.

The estimation of CaM in biological tissues has thus far been primarily accomplished by either enzyme-linked or radio immunoassay (RIA) procedures. Both of these methods have disadvantages which have made the elucidation of the role of CaM in both normal and pathological neurochemical processes difficult and/or ambiguous. The purpose of the present study was the development of a simple and efficient method for the determination of brain CaM levels using HPLC.

Rats were sacrificed by decapitation and brains were homogenized (1:5 w/v) in 15 mM Tris buffer (pH 7.4) containing 1 mM EGTA. Following heat inactivation (5 min at 90°C) and rapid cooling, samples were centrifuged at 10,000 xg for 30 min and the resulting supernatants stored at -20°C for analysis. Aliquotes from these supernatants were used to determine CaM by an RIA procedure and by HPLC. The HPLC system utilized an I-125 protein column coupled to an absorbance detector set at a wavelength of 214 nM. The mobile phase was 50 mM Tris buffer (pH 7.4) and was run at 0.5 ml/min.

Results indicate that brain CaM can be separated and quantified by HPLC. The assay is linear with respect to both CaM and protein concentrations and the specificity and validity of this assay for CaM is demonstrated by parallel radio-immunoassay determinations which give equivalent results.

In summary, a simple, specific, sensitive, inexpensive and efficient HPLC procedure for the determination of CaM levels in brain is presented. This method eliminates the need for radioligand, specific antibody preparation or lengthy and cumbersome preparation and/or incubation procedures.

328.2 RELEASE OF (^{3}H)-ADENOSINE FROM RABBIT RETINA. M.T.R. Perez* and B. Ehinger. Department of Ophthalmology, Research Unit B University of Lund, S-221 85 Lund, Sweden.

The release in vitro of (^{3}H)-adenosine, (^{3}H)-methyl-phenylethyl-adenosine and (^{3}H)-cyclohexyladenosine from the retina of light-adapted rabbits has been analyzed. Autoradiographic studies have shown that certain retinal neurons in the rabbit selectively accumulate (^{3}H)-methyl-phenylethyl-adenosine and (^{3}H)-cyclohexyladenosine and that some neurons and glial cells in the rabbit retina take up (^{3}H)-adenosine.

For the release studies pigmented rabbits were injected intravitreally with the different compounds. After 4 hours the eyes were enucleated and the posterior segments were placed in glass chambers where the retinae were superfused in the dark with a balanced salt solution. After 30 minutes the retinae were stimulated with flashing light or with 10 to 40mM K^+ at 37°C, 4°C and in a low Ca^{2+} perfusion medium. The collected superfusate was analyzed by liquid scintillation spectrometry in order to determine the amount of radioactivity released.

Light stimulation did not affect the release of any of the compounds. Elevated K^+ concentration resulted in significant increase of radioactivity for all substances. The K^+-induced release was also found to be temperature dependent and sensitive to changes in the Ca^{2+} levels.

328.3 AUTORADIOGRAPHIC LOCALIZATION OF ADENOSINE UPTAKE SITES IN RAT BRAIN USING [^{3}H] NITROBENZYLTHIOINOSINE. J-C BISSERBE,* J. PATEL* and P.J. MARANGOS. Biological Psychiatry Branch, NIMH, Bethesda, Md. 20205.

Ample evidence is now available suggesting a neuromodulatory role for adenosine in the peripheral and central nervous system. In common with other neurotransmitters, adenosine is stored within nerve terminals and is released from these structures in a calcium dependent manner. The released adenosine is believed to mediate its action by interacting with specific adenosine receptors. Two such classes of adenosine receptors (receptor A-1 and A-2) have been described in the past few years and have since been extensively characterized.

The actions of adenosine are terminated by removal of adenosine from the synaptic cleft. This is believed to be achieved largely as a result of its reuptake into the synaptic terminals. The reuptake of adenosine occurs via an energy dependent nucleoside uptake system, which has recently been demonstrated can be specifically labelled using tritiated nitrobenzylthioinosine (NBI) as a ligand. NBI, is one of the most potent inhibitors of adenosine uptake. Previously, using rat brain membranes, we demonstrated that neuronal tissue is enriched with NBI binding sites and that these sites are distinct from the adenosine type A-1 and A-2 receptors, and that dipyridamole, hexobendine and dilazep are the most potent (10^{-9}) inhibitors of binding in human brain.

In the present study, we demonstrate that specific 3H-NBI binding can be obtained using ultrathin rat brain slices which exhibit properties similar to those obtained with rat brain membrane. Furthermore, by performing authoradiography, we are able to describe the precise localization of NBI binding sites in the brain. High 3H-NBI receptor density were found in pyriform cortex, the septum, the nucleus accumbens, the caudate putamen, some nuclei of the thalamus, the superior colliculus, the dorsal tegmentum area, the central gray and substantia nigra. The highest density was observed in the nucleus of tractus solitarius. By using sequential slices for 3H-NBI or 3H-CHA (adenosine receptor A-2 ligand) binding, we have been able to compare the distribution of the adenosine uptake and adenosine receptor sites in the rat brain.

328.4 MICROMOLAR-AFFINITY ADENOSINE RECEPTORS IN BRAIN: IDENTIFICATION AND CHARACTERIZATION J.H. Chin* and R.J. DeLorenzo (SPON: J.R. Cooper) Dept. of Neurology, Yale University School of Medicine, New Haven, CT

Adenosine is released in micromolar concentrations from electrically-stimulated brain tissue. Adenosine, 2-chloroadenosine, and related purine compounds, in micromolar concentrations have profound depressant actions on central neurons. These effects are believed to result from presynaptic inhibition of neurotransmitter release possibly through a reduction in calcium availability. Recently, micromolar concentrations of adenosine have been shown to inhibit calcium spikes in hippocampal slice and superior cervical ganglion (Proctor, W.R. and Dunwiddie, T.V., Neurosci. Lett. 35: 197-201, 1983: Henon, B.K. and McAfee, D.A., J. Physiol. 336: 607-620, 1983). Although micromolar concentrations of adenosine also stimulate adenylate cyclase via A2-adenosine receptors, increases in cAMP do not correlate with the physiological actions of adenosine on neuronal firing, suggesting the existence of additional micromolar-affinity adenosine receptors and/or additional A2-receptor functions.

We have studied the binding of 2-chloro[^{3}H]adenosine, a stable analog of adenosine, to rat brain membranes and have identified a micromolar-affinity adenosine binding site with K_D = 4.9 uM and B_{max} = 66 pmol/mg as determined by Scatchard analysis. A number of adenosine analogs compete for binding of 2-chloro[^{3}H]adenosine with an order of potency 2-chloroadenosine > adenosine > 5'-AMP > cAMP > 2'deoxyadenosine > adenine > hypoxanthine > inosine. The methylxanthines inhibited binding with an order of potency 8-phenyltheophylline > isobutylmethylxanthine > theophylline > caffeine, paralleling their potencies as antagonists of adenosine actions on nerve cell firing. Binding was sensitive to both boiling and trypsin. This report demonstrates the presence of a micromolar-affinity adenosine binding site in brain that may represent a physiologically-significant central adenosine receptor. Further studies are necessary to determine the functional relationship of this receptor to the physiological and biochemical actions of adenosine.

328.5 EFFECTS OF MELATONIN ON ^{3}H-MUSCIMOL BINDING IN RAT BRAIN. F.M. Coloma* and L.P. Niles (Spon: E. Werstiuk).Department of Neurosciences, Faculty of Health Sciences, McMaster University, Hamilton, Ontario L8N 3Z5. Canada.

There is evidence that the centrally active pineal hormone, melatonin, which binds to specific, high affinity sites in the CNS (Niles, Soc. Neurosci. Abstracts 1983), competitively inhibits ^{3}H-diazepam binding in rat forebrain membranes (Marangos et al., Life Sci. 29, 259, 1981). Since diazepam acts on the benzodiazepine-γ-aminobutyric acid (GABA) receptor-ionophore complex to enhance GABA binding, we have investigated the possibility that melatonin may also act on this complex to modulate GABA binding in rat brain.

Aliquots of crude synaptic membrane suspensions were incubated with 2.5 nM ^{3}H-Muscimol and melatonin in varying concentrations at 0°C for 30 minutes. The samples were vacuum-filtered over Whatman GF/C filters and incubation tubes and filters were rapidly rinsed with 4x3 ml aliquots of ice-cold 0.05 M Tris-citrate or Tris-HCl buffer (pH 7.1). Specific binding of ^{3}H-Muscimol was defined as total bound radioactivity minus that not displaced by 10^{-4} M of GABA.

Preliminary studies indicate that melatonin, in concentrations ranging from 10^{-9}-10^{-5} M is capable of enhancing ^{3}H-Muscimol binding by about 20-40%, however, this effect is not seen consistently with Tris-citrate buffer. The use of Tris-HCl produced more consistent results and KCl (50 mM) appeared to enhance the effects of melatonin but not that of diazepam, suggesting that the effects of melatonin may be Cl^- ion dependent.

Melatonin(M)	Spec. Binding (%)	Diazepam(M)	Spec.Bind.(%)
none	100	none	100
10^{-7}	120	10^{-7}	147
10^{-7}+50mM KCl	156	10^{-7}+50mM KCl	121

These preliminary findings suggest that melatonin, like diazepam, is capable of enhancing ^{3}H-Muscimol binding in vitro. Future studies will be directed at determining whether melatonin's modulatory effects involve interaction at the benzodiazepine binding site or some other site on the benzodiazepine-GABA receptor-ionophore complex.

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328.6 EFFECT OF FORSKOLIN ON PROTEIN PHOSPHORYLATION AND INTRACELLULAR FREE CALCIUM IN PC12 CELLS. Carolyn S. Rabe and Forrest F. Weight Lab of Preclinical Studies, Nat'l. Inst. on Alcohol Abuse & Alcoholism, Rockville, MD 20852.

Incubation of PC12 cells with forskolin, an activator of adenylate cyclase, results in elevation of cellular cAMP levels and enhancement of depolarization-dependent neurotransmitter release (J. Cyclic Nuc. Res. 8:371, 1982). We have investigated the possibility that changes in protein phosphorylation and/or intracellular free Ca^{++} may be associated with the actions of forskolin in PC12 cells. Forskolin-induced changes in protein phosphorylation of intact PC12 cells were characterized by labeling ATP stores with ^{32}P-phosphate (0.3 mCi/ml) for 45 min. Incorporation of ^{32}P-phosphate into protein was analyzed using 2-dimensional gel electrophoresis and autoradiography. In the absence of forskolin, a large number of proteins were phosphorylated. Incubation of the cells with 1 uM forskolin produced significant changes in the phosphorylation of four approximately 17,000 dalton proteins with different isoelectric points. ^{32}P-phosphate incorporation increased in 3 of these proteins. It decreased, however, in the most basic of the M_r=17,000 proteins. These changes in phosphorylation were mimicked by incubation of the cells with 1 uM adenosine, another agent which both elevates cellular cAMP levels and enhances depolarization-dependent transmitter release. In contrast, incubation of the cells with 2 mM carbachol (which causes transmitter release) did not produce these changes in phosphorylation. Forskolin-mediated effects on intracellular free Ca^{++} were studied using the intracellularly trapped fluorescent Ca^{++} indicator, Quin-2. In the absence of forskolin, cells incubated in Hepes buffered saline containing 1.8 mM Ca^{++} had an estimated resting free Ca^{++} level of 119 nM. Addition of carbachol to a final concentration of 2 mM caused a 30% increase in these levels within 15 sec. Cells exposed to 1 uM forskolin had no detectable increase in resting free Ca^{++} when monitored over a 12 min period. When forskolin treated cells were stimulated with carbachol, no additional increase in free Ca^{++} above that seen in control carbachol stimulated cells was detected. The results suggest that the actions of forskolin in PC12 cells may be associated with alterations in protein phosphorylation but no apparent change in the mean intracellular free Ca^{++}.

328.7 L-DOPA PRETREATMENT COUNTERACTS THE "NORMALIZATION" OF BEHAVIOR PRODUCED BY METYRAPONE TREATED HIPPOCAMPALLY-LESIONED RATS. J.P. Ryan, J.E. Springer, J. Johnston,* R. Boccanfuso,* L. Glazer,* T. Shamash,* R.L. Isaacson. Dept. of Psychology and Center for Neurobehavioral Sciences, SUNY-Binghamton, Binghamton, NY 13901.

A temporary "chemical adrenalectomy" can be induced by metyrapone, a drug that blocks the synthesis of corticosterone at the 11-β-hydroxylation step. Ryan et al. (Neurosci. Abst., 1983) reported that the treatment with metyrapone decreased the hyperactivity of hippocampally-lesioned animals to the activity level of control animals. The decreased hyperactivity can be reduced or eliminated through pretreatment with corticosterone.

In this experiment, we investigated the hypothesis that the actions of corticosterone that alter activity levels in hippocampally-damaged animals may be their modulation of the biogenic amines. We chose to examine the interaction of dopamine (DA) with corticosterone in hippocampally-lesioned rats for two reasons: (1) corticosterone has been found to increase dopamine turnover in the brain (Iuvone et al., Brain Research 120: 545, 1979), and the hyperactivity of hippocampally-lesioned animals appears to involve alterations in dopamine activity (Springer & Isaacson, Brain Research 25: 182, 1982). We reasoned that if the suppression of corticosterone synthesis by metyrapone produces its effects by actions on the ascending DA systems then the beneficient effect could be circumvented through pretreatment with the dopamine precursor, L-DOPA.

Rats were prepared with sham, neocortical, or hippocampal lesions by aspiration. The animals were tested in an open field apparatus with metyrapone (25 mg/kg) only, or L-DOPA pretreatment 30 min before the administration of metyrapone or its vehicle. Metyrapone alone drastically reduced the locomotor activity and rearing of hippocampally-lesioned animals. Pretreatment with L-DOPA followed by the vehicle for metyrapone increased the activity of the animals in all three groups; however, only the activity of hippocampal-lesioned animals remained high even when administered metyrapone.

The results indicate that hippocampally-lesioned rats pretreated with L-DOPA are minimally affected by the metyrapone-induced suppression of corticosterone. This suggests that corticosterone may be acting as a neuromodulator for dopamine activity and that hippocampal animals are more sensitive to this modulatory effect than controls.

328.8 EFFECTS OF ADENOSINE ANALOGS ON PHOSPHORYLATION OF SYNAPTIC MEMBRANE PROTEINS. J. B. Rosen,* C. J. Towns*, & R. F. Berman. (SPON: J. L. RAM). Biopsychology and Neuroscience Programs, Wayne State University, Detroit, MI 48202.

In addition to its role in many biological processes, adenosine may also act as a neurotransmitter in the brain. Its presumed mechanism of action is to presynaptically inhibit release of excitatory neurotransmitters. Adenosine receptors have also been described, with A_1 receptors labeled by nanomolar concentrations of l-phenyl-isopropyl-adenosine (l-PIA) and linked to decreased adenylate cyclase activity, and with A_2 receptors labeled with nanomolar concentrations of N-ethyl-carboxamide-adenosine (NECA) and linked to increased adenylate cyclase activity. Adenosine has been shown to inhibit total protein phosphorylation in striatal slices (Williams, Brain Res., 109:90, 1976) and the adenosine analog, 2-chloro-adenosine, has been shown to stimulate phosphorylation of synapsin I in facial nucleus slices (Dolphin & Greengard, J. Neurosci., 1:192, 1981). In the present study we investigated the effects of the adenosine analogs l-PIA and NECA on in vitro phosphorylation of synaptic membrane proteins isolated from rat cerebral cortex. Rats were decapitated, brains quickly removed, cortex dissected on ice, and synaptosomes isolated by centrifugation. The lysed synaptic membrane fraction was incubated for 2 min at 30° C. with 10 uM [^{32}P]-ATP, with or without cyclic-AMP, and various concentrations (10nM to 100 uM) l-PIA or NECA. Proteins were separated by SDS-polyacrylamide gel electrophoresis. Autoradiograms of the dried gels were analyzed for ^{32}P-incorporation. Low concentrations of NECA (10nM) stimulated phosphorylation of a phosphoprotein band at approximately 14-15K daltons, while 10 nM concentrations of either l-PIA or NECA decreased phosphorylation of phosphoproteins at 43K and 50K by approximately 50% and 30%, respectively. Higher concentrations of l-PIA and NECA (10 uM and 100 uM) stimulated phosphorylation of a phosphoprotein at 43K by approximately 10%. In addition, high concentrations of NECA (100uM), but not l-PIA, inhibited cyclic-AMP stimulated phosphorylation of a phosphoprotein at 55K (protein II). These data indicate that adenosine analogs can exert biphasic and selective effects on phosphorylation of synaptic membrane proteins depending upon structure and concentration. (Supported by NIH grant RR08167)

328.9 AN ENZYME ELECTRODE APPROACH FOR CHARACTERIZING AND QUANTIFYING ACETYLCHOLINESTERASE INHIBITORS OF THE ORGANOPHOSPHATE TYPE. S. Mainer*, F.C.G. Hoskin* and K.S. Rajan, IIT Research Institute, Chicago, IL 60616

An enzyme electrode consisting of DFPase attached to a fluoride ion electrode has been developed for the quantification of di-isopropyl-phosphorofluoridate (DFP), an acetyl cholinesterase inhibitor. In this system the enzyme hydrolyzes DFP to produce a fluoride ion which is then sensed by the electrode. The enzyme was prepared from squid ganglia and was attached to the tip of the electrode by using a dialysis membrane. Two different electrode combinations were used in this research. By using the enzyme electrode in conjunction with a Ag/AgCl reference electrode the total concentration of unhydrolyzed DFP and free F^- produced by non-enzymatic DFP hydrolysis was measured. By using the enzyme electrode in conjunction with a combination fluoride ion electrode as the reference, the "unhydrolyzed DFP" concentration was measured. The electrode responses ranged from 32 mV to 159 mV for the first system and from -100.6 mV to -22.3 mV for the second. The electrode responses were linear over the range of DFP concentration, i.e., $1x10^{-3}$M -$1x10^{-6}$M. The theoretical basis of the DFPase electrode and its possible application to other cholinesterase inhibitors of the phosphonofluoridate type will be discussed.

328.10 EXOGENOUS ATP MODULATES QUANTAL TRANSMITTER RELEASE AT THE CRAYFISH NEUROMUSCULAR JUNCTION. C.A. Lindgren and D.O. Smith. Dept. of Physiology, University of Wisconsin, Madison, WI 53706.

Stimulation of the excitor axon to the crayfish opener muscle (50 Hz, 1 minute) causes ATP release from the muscle. In the periaxonal space, ATP levels were estimated to rise by as much as 5 mM. One possible role for this released ATP may be to modulate transmitter release at excitor-opener nerve terminals. To investigate this, average quantal release, $\bar{m}$, was measured at single synaptic sites using focal extracellular electrodes at 4°C.

Addition of 0.5- and 5-mM ATP to the bath depressed $\bar{m}$ by 26.8% and 24.7%, respectively, at stimulation frequencies ranging from 0.5 to 10 Hz.

Two related compounds, adenosine and dibutyryl cAMP (dBcAMP), were also examined. Adenosine (1mM) had no effect on $\bar{m}$, ruling out the possibility that ATP exerts its effect by first being hydrolyzed to adenosine. Conversely, dBcAMP (4 mM) had effects similar to ATP, reducing $\bar{m}$ by 42.1%. Thus ATP may act by increasing cAMP, although this remains to be determined conclusively.

The ability of 5-mM ATP to depress $\bar{m}$ is dependent on the presence of glucose (10 mM) in the medium. When glucose was absent, 5-mM ATP occasionally increased $\bar{m}$ dramatically (up to 87%), remaining elevated for at least two hours. Since omission of glucose leads to a 53% depletion of neuronal ATP after three hours, one possible explanation for this facilitatory effect is that exogenous ATP enters the nerve and restores transmitter release by replenishing intraneuronal ATP content.

The ability of ATP to modulate miniature end-plate potential (m.e.p.p.) frequency and synaptic facilitation was also examined. Neither 0.5- nor 5- mM ATP had any effect on m.e.p.p. frequency. In contrast, 5-mM ATP increased synaptic facilitation due to paired pulses (35-ms interval) at 0.5 Hz by 48%.

We conclude that during periods of intense neuromuscular activity ATP is released from the muscle and limits the amount of transmitter released by the presynaptic nerve. The released ATP may also function in some cases to replenish depleted nerve ATP, thereby insuring synaptic readiness. Supported by NIH grant NS13600 and the Whitehall Foundation.

328.11 EFFECT OF LIGHT AND DARK ON RETINAL MELATONIN LEVELS AND DOPAMINE (DA) RECEPTOR NUMBER. R.C. Lucas*, J.S. Takahashi and M.L. Dubocovich. Dept. of Pharmacol. Northwestern Univ. Med. Sch., Chicago, IL 60611 and Dept. of Neurobiol. and Physiol., Northwestern University, Evanston, IL 60201.

Picomolar concentrations of melatonin inhibit the calcium-dependent release of DA from the rabbit retina, but not from the striatum (Dubocovich, Nature 306:782, 1983). The objective of this study was to investigate whether the light-dependent production of melatonin regulates the number of retinal DA receptors by modulating DA release in vivo. The number of DA receptors was assessed by specific ^{3}H-spiperone binding in washed rabbit retinal and striatal membranes (J. Pharmacol. Exp. Ther. 227: 592, 1983). Immunoreactive melatonin was measured as described by Rollag and Niswender (Endocrinol., 98: 482, 1976). Scatchard analysis of ^{3}H-spiperone binding defined with α-flupenthixol (10 μM) resulted in a Kd=0.62 ± 0.14 nM and a Bmax = 321.2 ± 54.5 fmol/mg protein (n=3) in the rabbit retina. Retinal melatonin levels were decreased from 341.0 ± 129.1 pg/mg protein (n = 4, midnight) to 38.7 ± 2.5 pg/mg protein (n = 4, noon). Single point measurements of specific ^{3}H-spiperone (0.53 ± 0.02 nM) binding defined with 10 μM α-flupenthixol or 10 μM apomorphine in the retina were slightly lower at noon than at midnight. However, binding defined with α-flupenthixol was significantly lower in retinal membranes from rabbits kept 1 week in constant light (117.7 ± 4.9 fmol/mg protein, n = 6), than in membranes from rabbits kept 1 week in constant dark (159.2 ± 6.7 fmol/mg protein, n=4, $p < 0.005$). Similarly, specific ^{3}H-spiperone binding defined with 10 μM apomorphine decreased from 191.2 ± 4.5 fmol/mg protein (n=4, constant dark) to 128.0 ± 9.4 fmol/mg protein, (n=4, constant light, $p < 0.001$). Decreases in binding (constant light) were also observed for other apomorphine concentrations (0.01 - 100 μM). Constant light did not decrease the rabbit striatal specific ^{3}H-spiperone binding defined with either 10 μM apomorphine or 3 μM α-flupenthixol. The low retinal levels of melatonin in vivo during light might increase DA release and cause the DA receptor down regulation observed in retinas from rabbits exposed to constant light. These results support the suggestion by Dubocovich (Nature 306:782, 1983) that the light-dependent production of melatonin modulates the activity of DA containing retinal neurons in vivo. Supported by USPHS EY 04788.

328.12 EFFECTS OF BRANCHED-CHAIN AMINO ACIDS ON CENTRAL NERVOUS SYSTEM CATECHOLAMINES. J. K. Stewart* (SPON: M. Fine) Dept. of Biology, Virginia Commonwealth Univ., Richmond, VA 23284

Chronic elevation in blood concentrations of the branched chain amino acids (BCAA) occurs in diseases in which there is a deficiency of the enzymes that metabolize the BCAA's and in catabolic conditions, such as diabetes and traumatic injury. Although there is evidence that high levels of the BCAA's reduce transport of the catecholamine precursor tyrosine into the brain, there is no direct evidence that chronic changes in availability of BCAA's modulate brain catecholamine concentrations or turnover rates. In this study, male Sprague Dawley rats were fed during daylight hrs, 0900-1700 hrs, and treated daily with either saline or a mixture of leucine, isoleucine, and valine (0.4 umol of each amino acid per g of body wt) during a time that dietary amino acids were available. The amino acid and control solutions were injected intraperitoneally either once daily at 1000 hrs for 4 days or three times daily at 1000, 1300 and 1600 hrs for 12 days. On the last day of the injections, two groups of the animals treated for 12 days were injected with the catecholamine synthesis blocker, alpha-methylparatyrosine (a-mpt, 250 mg/kg initial dose, 125 mg/kg booster dose), at 2 hr intervals. All animals were killed by decapitation between 1100 and 1230 hrs on the final day of treatment. Brain sections were dissected and frozen on dry ice. Catecholamine concentrations were measured by HPLC-EC. Administration of BCAA's 3 times daily for 12 days was associated with a reduction in steady state levels of norepinephrine (NE) in the hypothalamus, 1488 ± 64 ng/g, compared to control levels of 1260 ± 51 ng/g ($p < 0.02$, n=8 rats per treatment group). The average fractional depletion of NE at 4 and 6 hrs after a-mpt was also less in BCAA-treated animals (28.5%) than in control animals (43.1%), suggesting a reduced rate of turnover of NE in the hypothalamus of rats treated with BCAA's. There were no differences in either steady-state or a-mpt-depleted concentrations of dopamine and epinephrine in the hypothalamus of these rats and no differences in levels of any catecholamine in the pons. In contrast to the findings in rats treated for 12 days, 4 days of treatment with BCAA's had no effect on hypothalamic catecholamines. Although these findings suggest that one or more of the BCAA's modulate noradrenergic neurons in the hypothalamus, the effects of the BCAA's may be mediated by BCAA-induced metabolic changes in the brain or periphery.
Supported by NIH grant AM 31882-02

328.13 PRE- AND POSTSYNAPTIC EFFECTS OF ADENOSINE IN THE CA1 REGION OF RAT HIPPOCAMPUS IN VITRO. W.R. Proctor and T.V. Dunwiddie, Dept. of Pharmacology, Univ. of Colorado Health Sci. Ctr., and VA Med. Research Service, Denver, CO 80262.

Adenosine has a potent depressant effect on excitatory synaptic transmission in rat hippocampus, but the pre- and/or post-synaptic nature of this effect has not been firmly established. Using the rat hippocampal slice preparation, we previously reported that adenosine (10-100 uM) has a direct post-synaptic effect on the calcium spike threshold of CA1 pyramidal cells of TTX-treated slices (Proctor and Dunwiddie, Neurosci. Lett. 35:197, 1983). The depressant effect of adenosine perfusion on responses to locally applied glutamate (a putative transmitter onto pyramidal neurons) also suggests a post-synaptic component of adenosine action.

We now show evidence that the majority of adenosine depression on excitatory postsynaptic potentials (epsp's) on rat hippocampal CA1 pyramidal neurons is pre-synaptic. Adenosine has been reported to act by increasing K^+ conductance (and hence causing a small resting membrane hyperpolarization as well as reducing the epsp amplitude; Segal, Eur. J. Pharmacol. 79, 193, 1982; Haas, in press). In the present experiments, K^+ conductance was blocked with 2.5 M Cs^+ acetate filled intracellular recording electrodes. We then compared adenosine depression of epsp amplitudes (produced by Schaffer collateral and commissural fiber stimulation) to epsp's recorded using 2.5 M K^+ acetate filled electrodes. Simultaneous extracellular recordings of field epsp's were made to correct for changes in extracellular field potentials (usually the extracellular field epsp was approximately 10-30% of the intracellular response). There was no significant difference between the effect of adenosine on epsp's recorded from cells with K^+ acetate vs. Cs^+ acetate filled electrodes. For example, 50 uM adenosine produced depressions of 63±11% (K^+ Ac^-) and 68±8% (Cs^+ Ac^-).

Because the depressant effect of adenosine is unaffected by treatments which block K^+ conductance in the post-synaptic cell, it is unlikely that post-synaptic effects of this neuromodulator contribute in a major way to the depression of synaptic transmission which it elicits. Thus, the primary mechanism by which adenosine reduces synaptic transmission would appear to be pre-synaptic, most probably via a direct reduction in transmitter release.

Supported by DA 02702 and VA 394463116 (T.V.D.) and grant DA 07043 (W.R.P.).

328.14 PROCTOLIN AND OCTOPAMINE MODULATE SYNAPTIC TRANSMISSION AT THE SAME NEUROMUSCULAR JUNCTION IN MANDUCA SEXTA. G. K. Fitch*, L. W. Klaassen*, and A. E. Kammer, Kansas State University, Manhattan, KS 66506

The efficacy of synaptic transmission at some synapses can be influenced by compounds that are not the chemical transmitter. We have investigated modulatory roles of two structurally very different compounds, the biogenic amine octopamine and the pentapeptide proctolin, at the neuromuscular junction of a fast muscle in the hawkmoth, Manduca sexta. To determine how these effects change with development, dorsal longitudinal muscle preparations of both adult moths and pupae were used.

In developing moths (17 days since pupation) superfusion of 10^{-6} M octopamine causes a 60% increase in the amplitude of the excitatory junction potential (EJP) produced in the muscle in response to a single stimulation of its motor neuron. Application of proctolin (10^{-6} M) results in a 77% increase in the amplitude of the EJP. In adult moths, however, actions of the two compounds are different. In experiments performed in low calcium saline, the initial 19 mv EJP was elevated by proctolin to a value above threshold for an active membrane response, thus eliciting a contraction of the muscle fiber. Application of octopamine to adult moths under the same conditions resulted in no change in the EJP amplitude.

These results suggest that control of synaptic transmission at this neuromuscular junction is complex, at times possibly involving at least two compounds other than the transmitter, and that modulation of synaptic transmission changes as the animal develops. Supported by NIH grant NS19257.

328.15 Can estrogen alter neuronal responsiveness to putative amino acid neurotransmitters? Sheryl S. Smith, B.D. Waterhouse and D.J. Woodward, (SPON: C.M. Michael). Dept. of Cell Biology, Univ. of Texas Health Science Center, Dallas, Texas 75235

Evidence by other investigators (Heron et al., 1980) suggests that steroids may alter membrane microviscosity in CNS tissues by acting through non-receptor mechanisms. The purpose of this study was to test whether E2 could alter neuronal activity or responsiveness to iontophoretically applied amino acid neurotransmitters in an area not reported to contain E2 receptors. Such a region is the cerebellum, which was selected as a model system for these studies because it has been well characterized electrophysiologically. Extracellular activity of cerebellar Purkinje (P) neurons was recorded from halothane anesthetized, adult, ovariectomized rats using multibarrel glass micropipets. Spontaneous firing rate and responses of single units to microiontophoretic pulses (10s pulses every 40s) of GABA (10-50 nA), glutamate (GLUT, 3-40 nA) or norepinephrine (NE, 2-15 nA) were examined before, during and after iontophoretic (.25mM 17 B-estradiol hemisuccinate) or jugular i.v. (1000 ng/kg 17 B-estradiol) administration of E2. Spontaneous firing rate was increased during both i.v. (4/7 cells) and iontophoretic (7/11 cells) E2 application. Both modes of E2 administration resulted in a 2 to 3 fold increase in P cell excitatory responses to GLUT, independent of the direction of change in spontaneous firing rate. This effect was seen as early as one minute after iontophoretic application of E2 (5/7 cells) and 15-40 min. following i.v. E2 (5/6 cells). In all cases, recovery to the control level of response was not observed by 30-60 min. after E2 administration. In contrast to the effect of E2 on GLUT responsiveness, GABA-mediated inhibition of P cells was either antagonized (4/7 cells) or unchanged (3/7 cells) following E2 application. Initial results also suggest an effect of E2 on the routinely observed enhancement of GABA and GLUT by NE. In summary, these preliminary studies suggest the intriguing hypothesis that E2 may alter neuronal sensitivity to specific neurotransmitter substances within the cerebellar circuitry. Further studies are underway to determine the specificity and the loci of action of these phenomena. Besides direct membrane effects, these actions of E2 may be indirectly mediated through interactions with the cerebellar noradrenergic system. (Supported by grants MH09010 to SSS and AA3901, DA02338 and the Biological Humanics Foundation to DJW.)

328.16 EVIDENCE FOR THE OCCURRENCE OF LEUKOTRIENES (LT) IN THE CENTRAL NERVOUS SYSTEM AND FOR A NEUROENDOCRINE ROLE OF LT. J.Å. Lindgren, A. Hulting, S.-E. Dahlén, T. Hökfelt, S. Werner and B. Samuelsson. Departments of Physiological Chemistry, Physiology and Histology, Karolinska Institutet and Department of Endocrinology, Karolinska Hospital, Stockholm, Sweden.

Leukotrienes (LT) represent a recently discovered group of biologically active compounds formed from arachidonic acid via the lipoxygenase pathway. LT has so far mainly been localized in leukocytes and lung tissue. In the present study a possible occurrence of LT in the central nervous system was investigated as well as effects of LT on release of hormones from the anterior pituitary.

Slices from rat brain were incubated with the ionophore A23187 (5 μM) and arachidonic acid (75 μM). After extraction and purification of the incubation buffer, the lipid extract was subjected to reverse-phase HPLC. The products were detected by UV absorbance. Three peaks with elution times corresponding to injected standard of LTC_4, LTD_4 and LTE_4, respectively, were obtained. The material was further identified using radioimmunoassay (RIA) (LTC_4) and bioassay (LTC_4, LTD_4 and LTE_4).

Slices from various regions of the rat brain were incubated and LTC_4-like immunoreactivity was measured by RIA. In slices from the caudate nucleus it was shown that ionophore induced release of LTC_4 was dose dependent with a maximum at $5x10^{-6}$ M. The LTC_4 formation was markedly inhibited by the lipoxygenase inhibitor NDGA (30 μM).

In studies on dispersed rat anterior pituitary cells in culture, LTC_4 induced release of LH with a maximal effect at 10^{-12} M and of ACTH at 10^{-10} M. No effects were observed on prolactin and growth hormone release.

The present studies provide evidence that LTC_4 may act as a messenger in the central nervous system and in neuroendocrine events.

Supported by Swedish MRC (03X-217; 03X-6805; 04X-2887).

328.17 TRIFLUOPERAZINE SUPPRESSION OF EVOKED & SPONTANEOUS FIELD POTENTIALS IN ORGANOTYPIC HIPPOCAMPAL EXPLANTS. J. Fowler & S.M. Crain, Dept. of Neuroscience, Albert Einstein Coll. of Medicine, Bronx, N.Y.

Trifluoperazine(TFP), a phenothiazine derivative has been reported to be a relatively specific antagonist of the Ca binding protein, calmodulin, in in vitro binding studies(Levin&Weiss`76) & has been used to assess the role of the Ca-calmodulin system in cellular function. In cultured rat cardiac cells, TFP(IC50=15uM) reversibly inhibits spontaneous contractions, although reversal is not total(Klein`83). In rat cortical slices TFP(10uM) causes almost complete inhibition of K+-stimulated 14C-GABA release (de Belleroche et al.`82) & in synaptosomal preparations (15uM) partially inhibits depolarization induced transmitter release(DeLorenzo`82). In the hippocampal slice TFP(40uM) has been reported to inhibit long-term potentiation(Finn et al.`80). In the present studies, organotypic hippocampal explants(0.5-0.8mm thick, cultured on collagen-coated coverslips; Crain & Bornstein `74) from neonatal mice were used to assess the effects of TFP on spontaneous & evoked normal & epileptiform field potentials recorded from the CA 3/2 regions after 2-4 wks. in culture(Fowler & Crain`82) Stimulating electrodes were located in the dentate area(0.5 msec pulses, 5-20 uamps). Baseline amplitudes were assessed in Hank's balanced salt solution(BSS) & compared to those obtained in BSS + TFP(1-150uM). TFP caused a progressive depression of evoked & spontaneous field potentials at 50(7 of 10 cultures) & 150uM(7 of 7 cultures) during 15 min tests whereas at 1uM, evoked & spontaneous slow-wave activity was not significantly different from baseline values. In the 50&150uM groups evoked or spontaneous activity could not be detected during the 1/2 hr interval following return to regular BSS even at stimulus strengths much greater than used to elicit baseline evoked potentials or in 2 cultures with the addition of convulsant agents, e.g. bicuculline(2uM) or kainic acid(50uM).Preliminary tests with another phenothiazine, chlorpromazine(at 50uM), did not have the profound depressant effects of TFP and only partly attenuated the amplitude of evoked potentials(<25%). Further studies are required to determine if these potent depressant effects of TFP on hippocampal field potentials are related to specific antagonism of the Ca-calmodulin system, other receptor/enzyme systems(Roufogalis`82) or local anesthetic effects(Ritchie & Greengard`61). Supported by NIMH predoctoral fellowship(MH15788) to J.F. Tissue culture facilities were provided by Dr. M.B. Bornstein.

328.18 MODULATION OF I_{Ca} AND LATE K CURRENTS BY INTRASOMATIC INJECTION OF Ca-CALMODULIN DEPENDENT KINASE IN HERMISSENDA GIANT NEURONS. J. Acosta-Urquidi, J.T. Neary, J.R. Goldenring, D.L. Alkon, and R.J. DeLorenzo. Sect. Neural Systems, Lab. Biophysics, NINCDS-NIH, MBL, Woods Hole, MA 02543 and Dept. Neurology, Yale Univ. Sch. Med., New Haven, CT 06510.

Long term regulation of ionic channels by neurotransmitters, hormones and neuroregulators, mediated via Ca-calmodulin dependent protein kinases (Ca-CAMdPKs), might be involved in neuronal circuit plasticity and is therefore an attractive model for mechanisms of learning and memory. Previous voltage clamp studies (Soc. Neurosci. Abstr. 9:561, 1983 and Science, in press) reported reduction of early and late K-currents after iontophoretic injection of a species of Ca-CAMdPK, phosphorylase kinase, into type B photoreceptors and identifiable giant neurons of Hermissenda. A distinct Ca-CAMdPK, tubulin-associated calmodulin-dependent kinase (TACK), that phosphorylates tubulin and microtubule associated proteins as major substrates has been isolated and purified from rat brain cytoplasm (J. Biol. Chem. 258: 12632,1983). TACK may have functional significance in regulation of neuronal excitability and synaptic modulation (Fed. Proc. 41:2265,1982). We report that iontophoretic (± 9 nA, 3-4 min) injection of TACK into giant Hermissenda neurons produced complex effects on I_{Ca}, $I_{K(V)}$ and $I_{K(Ca)}$. Changes were only taken as significant if they were ≥ 30% over pre-injection values. For I_{Ca}: 6/16 cells showed a persistent (> 10 min) reduction (48.26% ± 9.6); 6/16 showed a transient (1-2 min) increase (91.12% ± 29.8) and 4/16 no significant effect. For $I_{K(V)}$: 5/14 cells showed a persistent reduction (47.4% ± 4.3); 3/14 a transient increase (35.8% ± 2.6) and 6/14 no significant effect. For $I_{K(Ca)}$: 13/24 showed a persistent reduction (49.3 ± 5.5); 3/24 a transient increase (52.3% ± 15.7) and 8/24 no significant effect. Identical control injections of the carrier solution produced no significant effects on I_{Ca} (7 cells), $I_{K(V)}$ (7 cells) and $I_{K(Ca)}$ (10 cells). Detectable levels of TACK, ejected by standard iontophoresis, were assayed with tubulin as substrate. Parallel biochemical studies revealed that TACK altered the level of ^{32}P incorporation in several Hermissenda neural proteins, and it has been shown that the Hermissenda nervous system contains endogenous protein kinases which can be stimulated by Ca (Soc. Neurosci.,1984). Further studies are needed to clarify the diverse modulatory effects of TACK on I_{Ca} and late K currents.

328.19 AUTORADIOGRAPHIC EVIDENCE FOR MULTIPLE CNS BINDING SITES OF THE ADENOSINE ANALOGUES CHA AND NECA.
K.S. Lee and M. Reddington. Dept. of Neuromorphology, Max Planck Institute for Psychiatry, Martinsried, W. Germany.

Adenosine is a potent neuromodulator in many regions of the central nervous system. Multiple receptor sites have been described for adenosine, primarily on the basis of the differential action of various adenosine analogues including N6-cyclohexyladenosine (CHA) and N-ethylcarboxamide adenosine (NECA). The present study examined the distribution of tritium labelled CHA and NECA utilizing light microscopic autoradiographic techniques. The distribution of 3H-CHA was similar to that previously described by several laboratories. The binding of 3H-NECA differed from that of 3H-CHA both qualitatively and quantitatively. The binding of 3H-NECA was displaceable in a dose-dependent manner by unlabelled adenosine analogues with the following relative potencies NECA: 2-chloro-adenosine (2-CAD) 1-phenylisopropyladenosine (1-PIA). These findings are in contrast to those observed with 3H-CHA binding in which the relative capacities to displace binding are: 1-PIA 2-CAD NECA. 3H-NECA binding was not uniformly inhibited by 1-PIA. That is, the binding in certain regions of the brain such as the CA1 region of the hippocampus was almost totally blocked by 1 micromolar 1-PIA while the binding in the stratum lucidium of CA3 of the hippocampus was relatively unaffected. This difference could be due to the capability of 1-PIA to inhibit binding at the high affinity A1 adenosine receptor while permitting binding at another, lower affinity site. It is possible that the 3H-NECA binding which is not inhibited by low concentrations of 1-PIA represents the distribution of A2 adenosine receptors in the CNS. This is supported by the observation that brain regions which exhibit high adenosine-sensitive adenylate cyclase levels, such as the striatum and nucleus accumbens also exhibit high amounts of 1-PIA-insensitive 3H-NECA binding. A more thorough examination is currently in progress to clarify the relationship between the 3H-NECA binding sites and the distribution of adenosine-sensitive adenylate cyclase. In conclusion, the present data provide autoradiographic evidence for multiple binding sites for the adenosine analogues CHA and NECA. These sites differ both in terms of their distributions and sensitivity to other adenosine analogues.

328.20 ARACHIDONATE LIPOXYGENASE ACTIVITY IN RAT BRAIN SYNAPTOSOMAL PREPARATIONS. H.L. White and D.K. Stine*. Dept. of Pharmacology, Wellcome Research Labs, Research Triangle Park, NC 27709.

Lipid peroxidation in brain has been associated with degenerative changes that may play a role in both normal aging and in the pathology of illnesses such as senile dementia, Parkinsonism, and epilepsy. Oxidative metabolism of arachidonic acid in brain may be of particular importance because this unsaturated fatty acid is a predominant component of certain phospholipids and is readily released during the phospholipase A2 activation that may accompany anoxic insults. In this study synaptosomal fractions from rat brain homogenates were obtained by differential centrifugation and resuspended in a HEPES-salt buffer. Incubation of these preparations with [^{14}C]arachidonate at 37°C, pH 7.4, resulted in a time-dependent formation of [^{14}C] products via lipoxygenase and cyclooxygenase pathways. The presence of a calcium-dependent 5-lipoxygenase pathway was observed using 3 different thin-layer chromatographic systems and was verified by the action of known lipoxygenase inhibitors. Products of 12- and/or 15-lipoxygenase were also observed, as were cyclooxygenase products, PGD_2, PGE_2, $PGF_{2\alpha}$, and thromboxane B_2. [^{14}C]arachidonate was also incorporated into phosphatidic acid and other phospholipids.

When incubation mixtures were centrifuged before extraction of metabolites, most of the prostaglandins were recovered in the medium, while at least half the lipoxygenase products, most of the unmetabolized arachidonate, and all of the labelled phospholipids, including phosphatidic acid, were associated with the pellets.

Indomethacin (10μM) caused inhibition of cyclooxygenase product formation without significantly affecting the lipoxygenase pathway. Baicalein (10μM), a known lipoxygenase inhibitor, selectively blocked the formation of lipoxygenase products, as did EDTA (5 mM). BW 755C (100μM) inhibited both pathways and also decreased the incorporation of [^{14}C]arachidonate into phosphatidic acid and phosphoinositides without affecting incorporation of [^{32}P] inorganic phosphate into these metabolites. Continuing studies are directed toward understanding the significance of arachidonate lipoxygenase activity in normal and abnormal neuronal function.

RECEPTOR MODULATION II

329.1 CHANGES IN MEMBRANE FLUIDITY FOLLOWING CHRONIC STIMULATION OF THE PHOSPHATIDYLINOSITOL SYSTEM IN A SMOOTH MUSCLE CELL LINE. M.D. Dibner, K.A. Ireland*, E.E. Reynolds*, and B.B. Wolfe. Central Research & Devel. Dept., Du Pont Glenolden Lab, Glenolden, PA 19036 and Dept. of Pharmacol. Univ. of Penna. Medical School, Phila., PA 19104.

The DDT_1 smooth muscle cell line has a large phosphatidylinositol (PI) turnover response to stimulation at the α_1-adrenergic receptor. In these studies, cell surface membrane fluidity was measured in cells following chronic exposure to norepinephrine (NE). Fluidity was determined as the lateral diffusional recovery of the fluorescent lipid probe DiI following photobleaching in a laser-based fluorescence photobleaching system. In parallel studies, PI response, as measured by the NE-stimulated accumulation of (^{3}H)-inositol-1-phosphate (I-1-P) and α_1-adrenergic receptors (measured by ^{125}I-BE2254 binding) were studied. Exposure of cells to NE (10 μM) for 1.5 hr at 37°C led to a 20% increase in membrane fluidity. Concurrently, there was a 35% decrease in the I-1-P response to NE with no change in α_1-receptor density. Following 24 hr exposure to NE, both the membrane fluidity and I-1-P response changes were twice those seen at 1.5 hr. There was also a 35% decrease in α_1-receptor numbers (with no change in the receptor affinity for the ligand). Incubation of cells for 48 hr with LiCl (10 mM), which inhibits I-1-P phosphatase, led to a decrease in membrane fluidity by 50%. In contrast, the rat C6 glioma cell does not express a PI system response to NE. Exposure of these cells to NE or LiCl for 48 hr did not alter membrane fluidity. Thus, there is a correlation between changes in fluidity and chronic stimulation of the PI system. Whether a component of the PI system is responsible for the alterations in fluidity remains to be elucidated.

329.2 ALTERATIONS IN GUANINE NUCLEOTIDE MODULATION OF AGONIST BINDING BY LOW PH TREATMENT. S.P. Baker and S.R. Childers. Dept. of Pharmacology, Univ. of Florida, College of Med., Gainesville, FL 32610.

Guanine nucleotides modulate agonist binding to a variety of neurotransmitter receptors. Previous studies (Childers et al., Life Sci. [1983] 33, 215) showed that pretreatment of brain membranes at pH 4.5 increased GTP modulation of opiate receptors. We now report on effects of low pH pretreatment on muscarinic cholinergic and β-adrenoreceptors in heart and brain membranes. Muscarinic receptors and β-adrenoreceptors were measured with (-)-[^{3}H]quinuclidinyl benzilate (QNB) and (-)-[^{125}I]iodocyanopindolol (CYP) respectively. Agonist binding was determined by [^{3}H]oxotremorine-M (Oxo-M) binding and carbachol competition assays for muscarinic receptors and by isoproterenol competition assays for β-adrenoreceptors. Pretreatment of heart membranes at pH 4.5 for 20 min at 25°C did not affect the concentration of β-adrenoreceptors whereas the muscarinic receptor concentration was reduced by 17%. In control heart membranes, Gpp(NH)p increased the IC_{50} values for both carbachol and isoproterenol by 17-fold. After treatment at pH 4.5, Gpp(NH)p induced only a 3-fold increase in the isoproterenol IC_{50} value and had no effect on the carbachol IC_{50} value. In addition, pH 4.5 treatment completely abolished Oxo-M binding. In the brain, pH 4.5 treatment had no effect on the concentration of either receptor. Similar to the heart, pretreatment of cerebellum membranes abolished the ability of Gpp(NH)p to reduce the isoproterenol IC_{50} value. In contrast however, pH 4.5 pretreatment of cortical membranes increased the Gpp(NH)p induced shift of the carbachol IC_{50} value from 2-fold in control membranes to 9-fold in the treated preparation. Furthermore, the ability of Gpp(NH)p to maximally inhibit Oxo-M binding was increased from 48% in control membranes to 80% in pH 4.5-treated membranes.

These results suggest that in brain membranes low pH pretreatment may increase receptor-N protein (R-N) interactions for inhibitory cyclase systems and decrease such interactions for stimulatory cyclase systems, while R-N interactions are decreased for both systems in heart membranes.

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329.3 ALTERATIONS IN NONADRENERGIC RECEPTOR SENSITIVITY AND BEHAVIOR INDUCED BY CHRONIC IN-VIVO INFUSION OF FORSKOLIN. R. G. Browne and P. D. Suzdak, Pfizer Central Research and Dept. of Pharmacology, Univ. Connecticut.

Forskolin, a diterpene isolated from the plant coleus forskohlii, has been shown to activate the catalytic subunit of adenylate cyclase in vitro (Seamon and Daly, 1981, 1983), resulting in a hormone-receptor independent increase in the intracellular production of cyclic AMP. While studies to date have utilized Forskolin in in-vitro models, this study was undertaken to assess the effect of chronic infusion of Forskolin on noradrenergic receptor sensitivity and changes in behavior. Forskolin was infused at 6 or 12 ug/ul/hr into the right lateral ventricle of male Sprague Dawley rats via Alzet osmotic minipumps for seven days. Chronic infusion of Forskolin resulted in a dose related decrease in norepinephrine stimulated adenylate cyclase in the limbic forebrain. In addition, the Bmax of 3H-DHA binding in the cerebral cortex and hippocampus was decreased to 40% of control in the cortex after the 12 ug/ul/hr infusion. There was no apparent change in the Kd values. Thus, Forskolin appears to down regulate beta adrenergic receptors, possibly due to changes in receptor phosphorylation and mobility (Lefkowitz, 1982) following chronic elevation of cyclic AMP. Additional studies examined the effects of chronic Forskolin on alpha receptor activity (3H-PAC binding), since these receptors appear to be negatively coupled to adenylate cyclase. The results show that chronic infusion of Forskolin caused a dose dependent increase in the Bmax for 3H-PAC (up to 153% of control). The significance of these opposing changes in noradrenergic receptor sensitivity induced by chronic Forskolin will be discussed in relation to the observed behavioral effects (forced swimming, locomotor activity) of chronic Forskolin infusion. The results of the studies suggest the possibility for a novel therapeutic approach to modulating receptor sensitivity, and that chronic infusion of Forskolin may be a useful model for studying the role of cyclic AMP in the control neuronal activity.

329.4 AGE AND OVARIAN HORMONES AFFECT RESPONSES OF IMIPRAMINE BINDING SITES TO CHRONIC ANTIDEPRESSANT TREATMENT IN FEMALE RATS. M. A. Wilson and E. J. Roy. Psychology Dept., Univ. of Illinois, Champaign, IL 61820.

Decreased levels of binding sites for the antidepressant imipramine are associated with clinical depression. Effective therapies may increase the concentrations of imipramine binding sites. Animals studies, however, indicate that chronic treatment with antidepressants results in either a reduction in imipramine binding or no change. We have found that the response to antidepressant treatment differs qualitatively in animals of different ages, possibly due to age-related changes in drug metabolism.

We examined the interaction between age and chronic antidepressant treatment on imipramine binding in female rats. Females were treated with 10 mg/kg imipramine (IMIP) or saline (CONT) twice daily for 10 to 20 days until animals were 1.5 months old (JUVENILE), 3.5 months (YOUNG), or 14-17 months (MIDDLE-AGED). The level and affinity of imipramine membrane binding sites in the hypothalamus-preoptic area (HPA) were then determined using saturation analysis.

In juvenile females IMIP treatment induced a decrease in levels of imipramine binding sites. This decrease in imipramine binding sites associated with chronic IMIP treatment is dependent on ovarian hormones. Ovariectomy prevented the reduced levels of imipramine binding observed following IMIP treatment in juvenile animals. In young females IMIP treatment produced no change in the levels of imipramine binding. In middle-aged females IMIP treatment induced an increase in imipramine binding sites. K_d values of imipramine binding were significantly higher in HPAs from IMIP treated young and middle-aged females compared to age-matched CONT values. This change in affinity, and the concominant change in B_{max} of imipramine binding sites results from tissue retention of imipramine metabolites after IMIP treatment in older females, which act as competitor in the binding assay. CONT values demonstrated that age alters imipramine binding sites in the absence of antidepressant treatment. Older animals had increased levels of imipramine binding along with a decrease in affinity. Analysis of variance revealed a significant ($p<0.001$) main effect of age and a significant ($p < 0.001$) interaction between age and IMIP treatment on both the level and the affinity of imipramine binding. The results suggest that age affects the metabolism of imipramine which indirectly alters the observed response of imipramine binding sites to chronic antidepressant treatment in rats. (Supported by MH33577(NIMH) & PHS-5-T32 GM07143)

329.5 **DETECTION OF AMPHETAMINE-INDUCED CHANGES IN STRIATAL CALMODULIN WITH A SIMPLE, INEXPENSIVE, RADIOIMMUNOASSAY.** J.M. Roberts-Lewis, M. J. Welsh* and M. E. Gnegy. Departments of Psychology, Anatomy and Pharmacology, University of Michigan, Ann Arbor, MI 48109.

We describe here a procedure for the detection of nanogram quantities of calmodulin (CaM) in crude brain homogenates using sheep anti-CaM serum. The method is basically a modification of previously described CaM radioimmunoassays (Chafouleas et al., J. Biol. Chem., 254, 1979; Wallace and Cheung, J. Biol. Chem., 254, 1979), with the novel advantage of replacing protein A or second antibody procedures with a polyethylene glycol precipitation of antigen- antibody complex, thereby reducing time and reagent costs.

We used this assay to measure changes in the total levels and subcellular distribution of CaM in striatal homogenates from rats chronically treated with amphetamine (AMPH) compared to acutely treated animals. Chronic neuroleptic administration, which blocks dopamine (DA) receptors and results in the up-regulation of striatal DAergic function, increases the total amount of CaM bound to striatal membranes. Conversely, acute treatment of animals with AMPH, which releases DA, and results in a subsensitivity of DA receptors, increases the cytosolic CaM content. AMPH and neuroleptics have opposite behavioral effects after acute administration, but paradoxically, chronic treatment of animals with either drug results in a behavioral supersensitivity to AMPH. In order to examine possible differences in the striatal CaM distribution between acute versus chronic AMPH, we measured the total CaM present in cytosolic and 27,000 x g particulate fractions of striatal homogenates from rats treated with repeated injections of AMPH (2.5 mg/kg I.P. daily for 5 days) compared to a single AMPH administration. Boiled, crude tissue samples were incubated for 18 - 24 hours at 4 ^{0} C with sheep anti-CaM serum and approximately 10,000 cpm of [^{125}I]CaM. The incubation was terminated by adding one ml of a 15% polyethylene glycol solution to each tube. After centrifugation at 5,000 x g for 15 min., supernatants were aspirated and pellets counted for [^{125}I].

Our data suggest that repeated, as compared to acute, injections of AMPH lead to a significant decrease in the total levels of striatal CaM (425 ± 24 and 523 ± 24 µg CaM/g tissue wet wt., respectively), with no change in the relative distribution of CaM between the membrane and cytosolic fractions. This suggests differential mechanisms for the potential role of CaM in mediating supersensitivity induced by chronic neuroleptic treatment versus that produced by chronic AMPH. The CaM radioimmunoassay described here is a simplified and effective method that is sensitive to alterations in CaM levels in a discrete brain region resulting from in vivo pharmacological treatment.

Supported by NIMH grant 36044-03.

329.6 APPARENT LIGAND-MEDIATED INTERNALIZATION OF ANGIOTENSIN RECEPTORS IN RAT BRAIN MEMBRANES. J.B. Erickson, R.H. Abhold, and J.W. Harding, Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164-6520.

We examined the possibility that internalization or down-regulation of the angiotensin receptor may in part explain the complex kinetics of angiotensin binding to rat brain membrane. Radioligand binding using ^{125}I-Sar^1-Ile^8-AII and purified P_2 tissue fractions, previously incubated with varying concentrations of AII (10^{-9} to 10^{-5}M) and extensively washed to assure complete dissociation of bound AII, exhibited a 50 to 60% decrease at the higher pre-incubation AII concentrations. Utilizing a new rapid filtration technique, we appear to be able to separate membrane-bound ligand from ligand bound to a soluble protein or enclosed within vesicles. This technique employs a BSA-treated top filter and a polyethyleneimine (PEI)-treated bottom filter. Initial results suggest that membrane-bound receptors are trapped by the BSA filter, while soluble or vesiculated receptors pass through the BSA filter and stick to the PEI filter. This conclusion is supported by the observation that the ratio of PEI to BSA binding increases upon post-incubation sonication of tissue incubates prior to filtration. No such change in the binding ratio occurs if the tissue is sonicated prior to incubation with ligand. This suggests that the soluble or vesiculated component, which specifically binds the ligand, is generated during the interaction of receptor and ligand, and does not merely result from the physical treatment of the tissue. This data is additionally supported by centrifugation studies which indicate that PEI-bound soluble or vesiculated receptors are not observed in the supernatant fraction of tissue incubates, but are readily released from the corresponding pellets by sonication in hypotonic medium. Although these results are clearly preliminary, they are consistent with internalization of receptor-ligand complexes. Such a phenomenon could explain the tachyphalaxis that accompanies the central administration of angiotensins.

329.7 IN VITRO INVESTIGATIONS OF THE POTENTIAL REGULATORY ROLE OF SULFHYDRYL GROUPS IN MOUSE BRAIN GLUCOCORTICOID RECEPTORS. C.L. Densmore, W.G. Luttge and S.M. Emadian. Department of Neuroscience, Coll. of Medicine, University of Florida, Gainesville, FL, 32610.

Glucocorticoid hormones have profound metabolic, neuroendocrine and behavioral effects in the mammalian brain that may be mediated through their interactions with high affinity intracellular receptors. The binding capacity of these receptors appears to be regulable in vivo by factors that interact directly with the receptor. The present study investigated the reversible oxidation-reduction of sulfhydryl groups on the receptor as a potential means of glucocorticoid receptor up- and down-regulation.

CD-1 mice were adrenalectomized and ovariectomized 5-8 days before perfusion, decapitation and removal of brain and other tissues. No losses in [^{3}H]dexamethasone (DEX) binding capacity were observed in unlabeled cytosol prepared in 20 mM HEPES (pH 7.6), 2 mM dithiothreitol [DTT], 20 mM disodium molybdate and 10% glycerol [w:v] for at least 4 hr even at 22°C. When prepared in the absence of DTT, however, brain cytosol exhibited a time and temperature-dependent loss in binding capacity (60% decrease after 4 hr at 22°C). This loss was reversed completely upon addition of DTT prior to incubation with [^{3}H]DEX. Similar results were obtained with liver and kidney cytosols, which, in contrast to previous findings, were more dependent on DTT added exogenously than brain cytosol. When unlabeled brain cytosol (±DTT) was pretreated with dextran-coated charcoal (DCC), both the binding capacity and thermal stability of the receptor were reduced markedly. Subsequent addition of DTT restored binding capacity fully, but stability at 22°C was only restored partially. Subsequent addition of molybdate was without effect. These results suggest that DCC removes DTT and probably other endogenous factors required for stabilizing glucocorticoid receptors. To determine whether this DTT-reversible inactivation was associated with changes in receptor size or shape, activated and inactivated unlabeled brain cytosol was applied to sucrose density gradients with or without DTT respectively. Fractions were subsequently incubated with [^{3}H]DEX in the presence of DTT prior to bound/free separation assays. No differences in the sedimentation properties of the two forms of the unoccupied receptor were found, suggesting that the inactivation-activation associated with sulfhydryl oxidation-reduction does not involve a major change in the conformation of the receptor.

329.8 DORSAL ROOT GANGLION CELLS INDUCE SUBSTANCE P BINDING SITES ON SPINAL CORD NEURONS IN VITRO. G.E. Handelmann, S. Fitzgerald*, and P.G. Nelson. Lab. of Developmental Neurobiology, NICHHD, NIH, Bethesda, Md. 20205.

An important issue in synaptogenesis is the development and regulation of postsynaptic neurotransmitter receptors. Evidence indicates that innervation or the presence of the appropriate neurotransmitter are not necessary for the appearance of receptors, although they may determine the distribution of receptors on the cell surface. The present experiments indicate that these factors may also influence the number of receptors expressed.

Substance P (SP) is an important neurotransmitter in sensory pathways. The dorsal root ganglion (DRG) cells are a major afferent source of SP in the spinal cord. Spinal cord neurons taken from embryonic mice and grown in tissue culture display binding sites for [125I]SP. The addition of DRG cells to the cultures at the time of plating increased the number of binding sites measured three weeks later, although DRG cells do not have measureable SP binding sites themselves.

EFFECTS OF DRG CELLS AND NGF ON SP BINDING TO SPINAL CORD CELLS IN CULTURE

	DRG	Spinal Cord		Spinal Cord +DRG	
		No NGF	NGF	No NGF	NGF
SP Specific Binding (fmol/ug protein)	0	4.5	5.3	12.9*	14.3*
SP Content (pmol/ug protein)	13.0	4.8	4.4	10.7*	16.7*#

*Different from Spinal Cord, p<.05
#Different from No NGF, p<.05

The increased binding may be caused by the higher concentration of SP present, although the addition of nerve growth factor (NGF), which enhanced SP concentrations, only slightly increased the SP binding.

The addition of DRG cells does not influence the number of neurons surviving. When examined by autoradiography, the increase in binding appears to be due to an increase in the number of sites per cell, rather than an increase in the number of cells expressing sites.

329.9 PRE VS POST-SYNAPTIC CHANGES IN SPINAL MONOAMINERGIC SYSTEMS AFTER SELECTIVE NEUROTOXIN LESIONS. K. Stauderman*, R.M. Ademe* and D.J. Jones. Depts. Pharmacology & Anesthesiology, The Univ. Texas Hlth. Sci. Ctr., San Antonio, TX 78284.

Alpha$_2$ adrenoceptors are known to exist in rat spinal cord. Presumably, these receptors are primarily located pre and post-junctionally on noradrenergic synapses. However, evidence is accumulating that pre-synaptic inhibitory alpha$_2$ receptors also exist on serotonin (5-HT) nerve terminals. The possibility that a portion of the spinal cord alpha$_2$ binding sites reside on 5-HT nerve terminals was investigated by examining radioligand binding changes after destruction of spinal 5-HT neurons with 5,7-dihydroxytryptamine (5,7-DHT) or noradrenergic (NE) neurons with 6-hydroxydopamine (6-OHDA). Alpha$_2$, alpha$_1$ and beta binding, [^{3}H]-5-HT and [^{3}H]-NE uptake were examined in the spinal cord 3-14 days after intracisternal injections of either 5,7-DHT (150 ug 45-60 min after 25 mg/kg desipramine, i.p.) or 6-OHDA (150 ug 45-60 min after 10 mg/kg fluoxetine, i.p.).

Ligand binding was determined at 22°C with [^{3}H]-p-aminoclonidine (alpha$_2$), [^{3}H]-prazosin (alpha$_1$) and [^{125}I]-iodocyanopindolol (beta). Neuronal uptake of [^{3}H]-5-HT (50 nM) and [^{3}H]-NE (50 nM) was measured at 37°C in crude spinal cord synaptsomes as described (Neurosci Abstr 9:569, 1983). Experiments showed that, as expected, 6-OHDA (plus fluoxetine) caused a 90-100% decrease in [^{3}H]-NE uptake while having no inhibitory effect on [^{3}H]-5-HT uptake. Thus, 6-OHDA administered after fluoxetine pre-treatment produces a very selective lesion of spinal cord neurons. Concomitantly, the B_{max} of alpha$_1$, alpha$_2$ and beta receptors increased, indicating post-synaptic locations for these 3 receptor subtypes at noradrenergic synapses. After 5,7-DHT (plus desipramine), [^{3}H]-5-HT uptake is depressed 90-100% while [^{3}H]-NE uptake is unchanged. Thus, 5,7-DHT administered after desipramine pretreatment produces a very selective destruction of spinal cord 5-HT neurons. Further, preliminary evidence indicates that while 5,7-DHT lesions produce no change in either alpha$_1$ or beta receptor binding, the number of lower affinity alpha$_2$ receptors appears to decrease after loss of 5-HT nerve terminals in the spinal cord. This data suggests the low affinity alpha$_2$ binding sites in spinal cord may be related to the presynaptic alpha$_2$ receptors already demonstrated on 5-HT nerve terminals in brain areas.

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329.10 EFFECTS OF ALTERNATE DAY ELECTROCONVULSIVE SHOCK ON ADRENERGIC RECEPTORS IN RAT BRAIN. D. Jones, R.M. Ademe, L.J. Stone* and A.L. Miller (SPON: E. Undesser). Depts. Anesthesiology & Psychiatry, The Univ. Texas Hlth. Sci. Ctr., San Antonio, TX 78284.

Chronic electroconvulsive shock (ECS) has been reported to produce, as do most antidepressants, "down" regulation of CNS beta-receptors (Bergstrom and Kellar, Nature 278: 464, 1979). These investigators found no changes in alpha$_1$ receptors with chronic ECS, but more recent reports have noted modest increases (Vetulani et al. Brain Res. 275:392, 1983). In these, and most other studies of ECS and neurotransmitter systems, the physical seizures, apnea and cardiac slowing were not prevented, so that cerebral hypoxia and oligemia probably occurred.

We studied rats anesthetized with methohexital, paralyzed with succinylcholine, ventilated with 97% O_2:3% CO_2, and protected from ECS-produced vagotonic cardiac arrest or slowing with atropine methyl nitrate. ECS was given via ear clips 8 min after anesthetic and succinylcholine. EEG recordings verified the occurrence of seizure activity and EKG showed no cardiac arrest or slowing. Six ECS treatments were given on alternate days. Rats were sacrificed by decapitation 24 hours following the last treatment. Brains were removed and dissected by hand prior to immediate analysis or freezing.

Chronic ECS did not alter [^{3}H]-norepinephrine uptake by synaptosomes prepared from cerebral cortex. The B_{max} of cortical beta receptors, determined with [^{125}I]-iodocyanopindolol as ligand, decreased from 168 to 83 fmoles/mg protein in ventilated rats given chronic ECS, as compared to a decrease from 129 to 89 fmoles/mg protein in non-ventilated rats given chronic ECS. B_{max} of beta receptors did not change in the cerebellum. In the ventilated ECS group, hypothalamic beta receptors were reduced from 117 to 67 fmoles/mg protein. There were no changes in K_d for beta receptors under either condition in any region. Alpha$_1$ receptors, measured by [^{3}H]-prazosin binding, were increased in cerebral cortex from 109 to 140 fmoles/mg protein in the non-ventilated ECS group and 93 to 116 in the ventilated ECS group. The data indicate that chronic ECS, when given under the same conditions as electroconvulsive therapy, produces "up" regulation of alpha$_1$ and "down" regulation of beta receptors. Moreover, the changes appear to be selective for particular regions and do not involve NE uptake alterations.

329.11 CHRONIC MORPHINE ADMINISTRATION INDUCES DOWN-REGULATION OF OPIOID RECEPTORS IN THE MOUSE CNS. R.T. McCabe*, R. Karler* and J.K. Wamsley (SPON: S.A. Turkanis). Depts. of Psychiatry and Pharmacology, Univ. of Utah, Salt Lake City, UT 84132.

Tolerance, occurring in response to chronic opiate administration, may involve alterations in the affinity and/or number of opiate receptors present. Autoradiographic techniques have been employed in the present study to establish if and where these changes occur in the mouse CNS as a result of chronic treatment with morphine. Animals received daily injections of morphine (100 mg/kg) ip for 21 consecutive days. Controls were given an equal volume of vehicle during the same time period. Both groups were exsanguinated on day 22, the brain tissues were removed and rapidly frozen on dry ice and individual sections were cut in a cryostat. Autoradiographic labeling of opioid receptors involved preincubating tissue sections in buffer containing GTP and NaCl to reduce the binding of any endogenous ligand. Then, the tissue sections were incubated in 0.17M Tris-HCl buffer containing 4nM [^{3}H]-dihydromorphine (DHM). Displacement of the specific binding was accomplished by the addition of 10^{-6}M naloxone into the incubation medium of adjacent sections. The labeled sections were dried and apposed to sheets of LKB Ultrofilm. After an exposure period, the films were developed and examined using computer-assisted microdensitometry. The mean range of receptor binding in the morphine-treated mice *was 35-65% lower than in the same areas of the vehicle treated* controls. This dramatic down-regulation was most apparent in such structures as the nucleus tractus solitarius, locus coeruleus, nucleus ambiguus, nucleus accumbens, amygdaloid nuclei, preoptic region, olfactory tubercle, vestibular nuclei, and caudate-putamen. A saturation curve was generated by incubating separate sets of serial tissue sections in varying concentrations of [^{3}H]-DHM and examining the grain density associated with the nucleus accumbens. Scatchard analysis of these data suggest that an alteration in the actual number of receptors (B_{max}) is responsible for the lower binding. These observations indicate that a down-regulation of opioid receptor binding has occurred in the mouse CNS due to chronic morphine treatment. These conclusions agree with some previous reports but are at variance with others. The role of species differences, treatment regimen, tissue preparation, and assay conditions in the determination of the results is under investigation.

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329.12 PHARMACOLOGIC EFFECTS OF MELATONIN ON ACUTE STRESS-INDUCED CHANGES IN BRAIN γ-AMINOBUTYRIC ACID LEVELS AND RECEPTORS. L.P. NILES. Department of Neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5, Canada.

There is evidence that the pineal gland may play a role in maintaining brain homeostasis in mammals. The pineal hormone, melatonin, produces antiepileptic effects in humans and experimental animals, however the underlying mechanisms await clarification. In order to determine whether melatonin's stabilizing effect on CNS activity may involve central modulation of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), the acute effects of melatonin on serum and brain GABA levels and binding sites were examined.

One hour following injection of saline or a pharmacologic dose (200 ug, i.p.) of melatonin, cerebellar GABA levels (as measured by radio-receptor assay) were enhanced in controls but remained normal in melatonin treated animals. A significant ($p<0.01$) increase in serum GABA levels was also found in saline-treated animals while melatonin caused a partial but significant blockade of this increase. The above effects were not evident two hours post injection and appeared to be due to a stress-induced rise in GABA levels as a result of the handling and injection of animals.

The affinity and concentration of cerebellar GABA receptors labelled by the tritiated agonist, ^{3}H-muscimol, *were decreased one hour after injection in saline-treated* as compared with melatonin-injected animals.

^{3}H-Muscimol Binding in Cerebellum 1 Hour After Injection

Treatment	K_d	B_{max}
Saline	6.5 nM	970 fmol/mg protein
Melatonin	3.4 nM	1130 fmol/mg protein

It is questionable whether the abnormal receptor binding seen in controls was primarily due to competitive inhibition by elevated residual GABA in brain membranes since the previously frozen tissues were treated with triton X-100 and washed prior to binding studies.

These findings suggest that melatonin is capable of attenuating the disruptive effects of acute stress on GABA levels and receptors by a presently unknown mechanism. (Supported by the Medical Research Council of Canada and the Ontario Mental Health Foundation.)

329.13 GABA AND BENZODIAZEPINE RECEPTOR CHANGES IN STRIATUM AND ITS PROJECTION AREAS AFTER MEDIAL FOREBRAIN BUNDLE LESIONS. H.S. Pan, A.B. Young and J.B. Penney. Univ. of Michigan, Dept. of Neurology, 1103 E. Huron, Ann Arbor, MI 48104.

The destruction of striatal GABAergic output cells induces GABA and benzodiazepine (BDZ) receptor up-regulation in globus pallidus (GP) and substantia nigra pars reticulata (SNr). Dopaminergic nigrostriatal neurons are thought to inhibit striatal output cells. The removal of the nigrostriatal input, therefore, should cause GABAergic striatal efferent neurons to be overactive and should induce GABA and BDZ receptor down-regulation in GP and SNr. Quantitative autoradiography was used to investigate GABA and BDZ receptor changes after 6-hydroxydopamine (6-OHDA) induced medial forebrain bundle (MFB) lesions.

Male Sprague-Dawley rats (145-150 g) were pretreated with desipramine 1/2 hr before 6-OHDA was pressure injected into MFB. Five months after the surgery, rats were killed. A slice of striatum was obtained from each rat for [^{3}H]dopamine uptake to evaluate the lesion. Only animals with a 90% or better reduction in uptake were used in binding assays. The rest of the brains were processed for autoradiography as described previously (Pan et al., J. Neurosci. 3: 1189-1198,1983). Prewashed tissue was incubated in various concentrations of [^{3}H]muscimol or [^{3}H]flunitrazepam (FLU) with or without various drugs. Dried sections were exposed to Ultrofilm ^{3}H (LKB). After a 12-14 day exposure, films were developed. Densitometry was then performed to convert optic densities to amount of radioligand bound.

Five months following 6-OHDA lesions, [^{3}H]muscimol binding was decreased in striatum (18%, $p<.01$, paired t-test) and GP (32%, $p<.02$), but increased in SNr (60%, $p<.01$). Similarly, [^{3}H]FLU binding was decreased in striatum (18%, $p<.05$) and GP (33%, $p<.01$), but increased in SNr (39%, $p<.01$). Scatchard analyses revealed the changes to be increases in Bmax. Preliminary data showed that the type I BDZ receptor ligand CL 218,872 (1 μM) did not alter the binding asymmetry of [^{3}H]FLU (7 nM) in GP and SNr. The data suggested that GABA and BDZ receptors are regulated differently in GP and SNr after MFB lesions. New interpretations of the striatonigrostriatal circuitry is needed to explain the differential receptor regulation. Furthermore, the trans-synaptic BDZ receptor changes may affect type I BDZ receptors less than type II receptors.

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329.14 REGULATION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS IN PC12 CELLS BY CHRONIC DEPOLARIZATION. E. M. DeLorme* and R. McGee, Dept. of Pharmacology, Georgetown Univ. Sch. of Med., Washington, D.C. 20007

In general, increased receptor occupancy by agonists results in a down regulation of the receptor. For the neuronal nicotinic acetylcholine (ACh) receptor, agonist activation produces cation flux via the receptor-linked ion channel resulting in local membrane depolarization. However, it has not been established whether this membrane depolarization is an integral step in the regulation of cellular responsiveness on a long-term basis. The effects of membrane depolarization were examined using pheochromocytoma cells, clone PC12, which possess neuronal nicotinic ACh receptors. We have previously shown that chronic exposure of the cells to an agonist causes a decrease in receptor-mediated response which appears to represent a decrease in receptor number. Chronic membrane depolarization was accomplished by elevating K^+ in the normal growth medium (Na^+ reduced to maintain osmolarity) for up to 14 days. To assess receptor function, agonist-induced uptake of $^{86}Rb^+$ was measured for 20 sec at 22°C in Na^+-depleted balanced salt solution containing ouabain to inhibit Na^+,K^+-ATPase. Initial experiments showed that agonist-induced uptake of $^{86}Rb^+$ was reduced while the cells were depolarized. These direct effects of depolarization were reversed by a 45 min repolarization process, allowing differentiation between short-term and long-term effects of depolarization. Exposure of the cells to elevated K^+ caused a time and concentration-dependent decrease in receptor responsiveness. Concentrations of K^+ between 30 mM and 70 mM produced decreases in responsiveness of 40%-70%. Changes were observed within 1 day and were maximized by 7 days. Upon removal of elevated K^+ from the culture medium cells returned to control within approximately 2-3 days. As a first step in determining if the properties of the receptors were the same after chronic depolarization, the concentration dependence for carbachol-induced uptake of $^{86}Rb^+$ was examined. No significant differences in the shape of the agonist dose response curve were seen, suggesting that the sensitivity of the remaining receptors was unaltered. The simplest explanation for the decreased responsiveness of the cells was that the number of ACh receptors had decreased. These results suggest that membrane depolarization may be an integral component in long-term regulation of the neuronal nicotinic ACh receptor. Supported by NSF grant BNS 82 16306 and NIH RCDA NS 00567.

329.15 INHIBITION OF ACETYLCHOLINE RECEPTOR MEMBRANE INCORPORATION IN THE ABSENCE OF EXTRACELLULAR CALCIUM. M. A. Wozniak* and R. J. Bloch. Department of Physiology, Univ. of Maryland School of Medicine, Baltimore, MD 21201.

The muscle cell line, BC3H-1, synthesizes and inserts nicotinic acetylcholine receptors (AChR) into its cell membrane. The rapid insertion and degradation of AChR, as assayed by specific ^{125}I-α-bungarotoxin binding, makes this cell ideal for studying factors which alter AChR metabolism.

In the presence of control (1.8 mM) extracellular calcium (Ca^{2+}_o), AChR appear on the cell surface at a linear rate. When BC3H-1 cells are cultured in medium containing 25 uM Ca^{2+}_o, or incubated with cycloheximide, AChR appearance is completely inhibited after an initial 2 hr lag. Transport of pre-existing AChR to the surface appears normal in the absence of Ca^{2+}_o. Half-maximal Ca^{2+}_o for AChR appearance is 100 uM. Other divalent cations (Ba^{2+}, Sr^{2+}, Mg^{2+}) do not substitute for Ca^{2+}. Reincubation of Ca^{2+}_o-deprived cells with 1.8 mM Ca^{2+}_o results in the reappearance of AChR on the cell surface after a 4 hr time lag. Reappearance is blocked by cycloheximide, but is unaffected by actinomycin D at a concentration which blocks 80% of ^{3}H-uracil incorporation into TCA-precipitable material. AChR degradation is unchanged during Ca^{2+}_o deprivation. Total protein and glycoprotein synthesis is only minimally decreased (10-20%) in the absence of Ca^{2+}_o. Autoradiograms of metabolically labeled protein extracts, separated by SDS-PAGE, indicate no major differences between control and Ca^{2+}o-deprived cells.

Decreasing Ca^{2+}_o preferentially and completely inhibits a step in AChR metabolism prior to or during AChR subunit synthesis. Metabolism of most other proteins or glycoproteins is only nonspecifically and mildly decreased.

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329.16 THE ROLES OF MEMBRANE DEPOLARIZATION AND PROTEIN GLYCOSYLATION IN THE REGULATION OF NEURONAL MUSCARINIC RECEPTORS. W. C. Liles* and N. M. Nathanson (SPON: A. Berger). Dept. of Pharmacology, University of Washington School of Medicine, Seattle, WA, 98195.

Cell cultures of N1E-115, a cloned murine neuroblastoma cell line, were employed to determine the effects of membrane depolarization on neuronal muscarinic receptors (mAChR). Veratridine (VTN), which causes a persistent activation of the sodium channel in excitable membranes, was used to produce prolonged depolarization of N1E-115 cell cultures. When added to confluent N1E-115 cultures, VTN induced a bipolar response in mAChR number. High concentrations (50 µM) of VTN consistently induced a reversible 50-100% increase in membrane mAChR density within 16 hrs as determined by the binding of the muscarinic antagonist ^{3}H-quinuclidinyl benzilate (^{3}H-QNB). This increase did not occur when protein synthesis was inhibited by cycloheximide (100 µg/µl). The VTN-induced increase in receptor number was partially inhibited by pre-incubation with tetrodotoxin, the specific sodium channel blocker. However, high external K^+ (70 µM)-induced depolarization also caused an increase in mAChR number, suggesting that membrane depolarization, rather than sodium channel activity per se, was responsible for the observed mAChR increase. Low concentrations (100 µM) of VTN, on the other hand, induced a 20-40% decrease in mAChR density. Studies are currently being conducted to determine whether Ca^{++} flux or cyclic nucleotides are involved in the regulation of mAChR in response to membrane depolarization.

The role of protein glycosylation in the maintenance of N1E-115 mAChR is also being studied. Tunicamycin (0.35 µg/ml), a potent inhibitor of protein glycosylation, reduced the macromolecular incorporation of ^{3}H-mannose by 75-80% when added to the media of confluent cultures, while ^{3}H-leucine incorporation remained 90-95% of the control value. When administered to growing cultures, tunicamycin reduced specific ^{3}H-QNB membrane binding to 75% of the control value after 36 hrs; comparison with cycloheximide-treated cultures indicated that tunicamycin did not abolish the synthesis of new mAChR. Furthermore, tunicamycin inhibited the return of mAChR by 25% following carbachol-induced down-regulation. Scatchard analysis demonstrated that tunicamycin treatment did not alter the K_D of mAChR for ^{3}H-QNB. The rate of disappearance following cycloheximide administration was not altered by tunicamycin treatment, suggesting that glycosylation does not affect the half-life of mAChR. Experiments are currently being conducted to determine whether glycosylation is involved in the insertion of mAChR into the cell surface plasma membrane.

329.17 DECREASED PHYSIOLOGICAL SENSITIVITY MEDIATED BY NEWLY SYNTHESIZED MUSCARINIC ACETYLCHOLINE RECEPTORS. D. D. Hunter* and N. M. Nathanson. Department of Pharmacology, University of Washington, Seattle, WA, 98195.

Treatment of chicken embryos in ovo for 8 hours with the muscarinic agonist carbachol results in an 85% reduction in the number of muscarinic acetylcholine receptors (mAChR) present in atrial membrane homogenates. Subsequent treatment of embryos with the muscarinic antagonist atropine results in a gradual increase in mAChR number, which returns to control levels after 14 hours. This recovery is blocked by administration of the protein synthesis inhibitor cycloheximide.

Measurements of the negative chronotropic response to applied carbachol with isolated atria show that even after recovery of receptor number to control levels, the response to agonist is diminished. The IC_{50} for carbachol is shifted approximately 10-fold from controls at 20 hours after atropine treatment, but less than 3-fold at 28 hours. This increase in physiological sensitivity is not blocked by cycloheximide. Receptors at 20 hours have binding constants for agonists and antagonists which are indistinguishable from controls. This implies that there is a defect in coupling of mAChR binding to the physiological response when mAChR reappear.

Recovery of the ability of mAChR to inhibit adenylate cyclase parallels recovery of the beating response; that is, the IC_{50} is shifted approximately 11-fold from controls at 20 hours after atropine treatment, yet only 3-fold at 28 hours. Thus, newly synthesized mAChR exhibit decreased physiological and biochemical responses to muscarinic agonists, suggesting that mAChR are initially synthesized in a less active form.

Preliminary results in primary culture of embryonic chick heart suggest that similar processes operate in vitro. Treatment of cardiac cultures with carbachol results in a 70% decrease in mAChR; removal of the agonist results in an increase in surface mAChR to control levels over 12 hours. Membrane homogenates of cells containing newly synthesized mAChR exhibit a decreased response to agonist in the inhibition of adenylate cyclase.

330.1 ACTH OR IMIPRAMINE TREATMENT MODIFY β-ADRENERGIC RECEPTOR COUPLING IN RAT BRAIN CORTEX. R.S. Duman*, S.J. Strada and S.J. Enna. Depts. of Pharmacol. and of Neurobiol. and Anat., Univ. Texas Med. Sch., Houston, TX 77025; and Dept. Pharmacol., Univ. South Ala. Sch. Med., Mobile, AL 36688.

While it is established that antidepressant or ACTH administration decreases norepinephrine (NE)-stimulated cAMP accumulation in rat brain cerebral cortex, little is known about which aspect of the receptor-coupled cyclic nucleotide system is most affected by these substances. A study was undertaken to address this issue by examining the influence of these treatments on the individual components of the cyclic nucleotide generating system in brain. Male-Sprague-Dawley rats were administered ACTH (50 IU/kg, s.c., once daily) or imipramine (10 mg/kg, i.p., once daily) for 7 or 21 days. Eighteen hrs after the last injection the animals were decapitated and the cerebral cortex used to study adenosine-and NE-stimulated cAMP accumulation and for preparing P_2 fractions to examine adenylate cyclase activity, phosphodiesterase (PDE) activity and the guanine nucleotide binding (G/F) protein. A 30%-40% decrease in NE-stimulated cAMP accumulation was noted following either 7 or 21 days of continuous treatment with imipramine or ACTH. However, adenosine-stimulated cAMP production was not influenced. Neither treatment altered Gpp(NH)p, flouride, or forskolin-activated adenylate cyclase activity in P_2 membrane fractions, nor were cAMP, cGMP or cGMP-stimulated cAMP PDE activities modified. G/F protein was extracted from brain membranes into cholate and its activity measured in the cyc- mutant of the S49 lymphoma cell. Flouride, Gpp(NH)p and isoproterenol-stimulated cAMP accumulation were completely restored in these cells in the presence of the brain extract whether or not the animals had been treated with the peptide hormone or antidepressant. These data indicate that neither ACTH nor imipramine treatments alter the activity of brain cyclase, G/F protein or PDE under conditions where NE-stimulated cAMP accumulation is reduced. This suggests these agents influence responsiveness by a selective action on the noradrenergic receptor recognition site. Given our earlier report that ACTH or imipramine treatments alter agonist affinity for adrenergic receptors, it is possible that these agents modify the coupling between the recognition site and G/F protein. This conclusion is reinforced by the finding that neither treatment affected adenosine-stimulated cAMP production, which would be expected if a change occured in a post-recognition site component. (Supportec in part by USPHS grant MH-36945).

330.2 COMBINED ELECTROCONVULSIVE SHOCK (ECS) AND TREATMENT WITH IMIPRAMINE (IMI) PRODUCES A RAPID DOWN-REGULATION OF CORTICAL BETA-ADRENERGIC RECEPTORS. I.A. Paul*, G. Duncan*, R.A. Mueller, and G.R. Breese (SPON: J. T. Wilson) Biological Sciences Research Center, UNC School of Medicine, Chapel Hill, NC. 27514.

Among the various antidepressant (AD) treatments, there is a considerable time lag between initiation of treatment and the onset of therapeutic effects. Typically, tricyclic AD's, MAO inhibitors, and atypical AD's require 2-3 weeks of continuous treatment to produce significant effects. ECS requires somewhat less time, between 10-14 days of treatment on alternate days. We have reported previously that forced swimming potentiates the effect of IMI, producing a rapid down-regulation of beta-adrenergic receptors in limbic forebrain (Duncan et al, Fed. Proc. 43:940). We now report that similar effects can be achieved with 4 days of concurrent ECS and IMI treatment.

Singly housed, male, Sprague-Dawley rats (Charles River), weighing between 200-250 g at the start of treatment were subjected to ECS [500 V (AC) @ 50 mA for 210 msec] administered via ear clips once daily for 4 days. Five minutes after each treatment, animals were administered saline, 5, or 10 mg/kg IMI, i.p. Animals were sacrificed 24 h after the last treatment and the brains were quickly removed over ice. Cerebral cortex, both striata, and brainstem were seperated and stored at -70° C until assayed with ^{3}H-Dihydroalprenolol according to the method of Bylund and Snyder (1976). Results of these assays using cortex are presented below:

Treatment	^{3}H-DHA Spec. Binding	N
Sham + Saline	1223 + 50	14
Shock + Saline	1203 + 84	5
Sham + IMI (5 mg/kg)	1121 + 46	8
Shock + IMI (5 mg/kg)	928 + 32*	6

*p< 0.01 when compared to other groups.

Similar changes were noted in rats given 10 mg/kg IMI + ECS for 4 days. The sham + 10 mg/kg IMI rats also showed significant reduction in binding compared to sham + saline.

These results suggest that ECS can potentiate the action of IMI and that this effect is more clearly apparent at low doses than at high doses. The effects of ECS and of IMI do not appear to be additive, since ECS + saline has no effect. Investigations are currently in progress to determine if this potentiation occurs at doses of IMI below 5 mg/kg. (Supported by HD-03110 and MH-36294).

330.3 EFFECTS OF CHRONIC ANTIDEPRESSANT DRUGS ON BRAIN PART BETA AND ^{3}H-IMIPRAMINE BINDING IN OLFACTORY BULB LESIONED RATS. J.A. Jesberger* and J.S. Richardson. Depts. of Pharmacology and of Psychiatry, College of Medicine, University of Saskatchewan, Saskatoon, SK S7N OWO

Research directed at understanding the therapeutic mechanisms of antidepressant drugs has been hampered by the lack of a selective animal model for investigating the brain substrates involved in the antidepressant activity. It has recently been reported that the rat olfactory bulbectomy model exhibits good predictive value as a screening procedure for antidepressant drug activity. The bulbectomized rat may have a pathological defect that is particularly sensitive to the action of clinically effective antidepressants. This model with its well defined behavioral syndrome was used to investigate some of the neurochemical effects of four different species of antidepressant drugs. The rats received daily injections of either saline, amitriptyline, mianserin, iprindole, or tranylcypromine for 28 days. After a 5 day drug-free period to insure clearance of residual drug from the brain, behavioral testing was performed, the rats were sacrificed by decapitation, and the brains were rapidly removed, dissected and stored at -80°C.

Olfactory bulb removal increased ^{3}H-dihydroalprenolol binding in the hippocampus and pons but caused no change in the midbrain and hypothalamus, and resulted in an increase in ^{3}H-imipramine binding in the midbrain, a decrease in the hippocampus and pons, and no change in the hypothalamus. In some cases these changes were returned to control levels by the chronic antidepressant drug treatment. However, the specific effects of each drug on beta and ^{3}H-imipramine binding varied between the 4 brain regions such that no two drugs had identical response profiles. This suggests not only that the different drugs are acting on different neurochemical substrates but also that the distribution and sensitivity of these substrates vary for different brain regions. While the identification of the specific neurochemical pathology in olfactory bulbectomized rats that is sensitive to the actions of an antidepressant drugs must await future research, the present results support the view that the olfactory bulbectomy model possesses relevant and attractive characteristics for studying the neurochemical mechanisms involved in antidepressant drug action. Supported in part by grants from SHRB and STRF.

330.4 COMPARISON OF [^{3}H]SULPIRIDE BINDING SITES BETWEEN CASTRATED AND SHAM OPERATED MALE RATS BY A QUANTITATIVE AUTORADIOGRAPHY TECHNIQUE. T. Ryan Jastrow* and M.E. Gnegy. Neuroscience Program and Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, MI 48109

Sex hormones influence neural dopaminergic systems at a variety of sites including the dopamine (DA) receptor. Increases in striatal DA receptors have been found following estrogen treatment. Our lab demonstrated that DA-sensitive adenylate cyclase (D_1 receptor activity) was less sensitive in striatal membranes from sexually immature or castrated (CAS) adult male rats as opposed to sham-operated (SHAM) male rats. Further, chronic treatment of rats with the D_2 selective drug sulpiride increased the sensitivity of adenylate cyclase for DA in striatal membranes from CAS but not SHAM rats. These findings suggest that sex hormones may affect the sensitivity and pharmacological profile of striatal DA receptors. To investigate sex hormone-related changes in striatal D_2 receptor characteristics we examined [^{3}H]sulpiride binding in CAS and SHAM male rats using a quantitative autoradiography technique.

Twenty-micron sections of rat brain were thaw mounted onto gelatin coated slides. Slides were incubated for 60 min at 22°C in 0.5 to 40 nM [^{3}H]sulpiride in 50 mM Tris-HCl pH 7.7 and 120 mM NaCl. Slides were then rinsed 2 x 5 min in ice cold 50 mM Tris-HCl pH 7.7, blown dry with warm air and apposed to tritium-sensitive LKB Ultro film for 40 days. Film was developed and optical densities were determined with computer-assisted microdensitometry. Radioactivity was determined by regression analysis, comparing film densities produced by sections with those produced by standards.

Equilibrium studies showed [^{3}H]sulpiride binding to be saturable and reversible. Scatchard analysis showed a single set of sites with a Kd of 3.2 nM and Bmax of 447 fmol/mg protein. Rate constants for association (k_1) and dissociation (k_{-1}) of 5 nM [^{3}H]sulpiride were $5.0 \times 10^{-7} M^{-1} min^{-1}$ and $1.4 \times 10^{-1} min^{-1}$, respectively. The Kd calculated as k_{-1}/k_1 was 2.8 nM.

Comparison of the D_1 binding characteristics of [^{3}H]sulpiride between CAS and SHAM adult male rats showed no significant difference in Kd or Bmax. CAS: Kd = 4.4 nM, Bmax = 386 fmol/mg P, SHAM: Kd = 4.2 nM, Bmax = 371 fmol/mg P. These results suggest that removal of sex hormones did not affect the binding characteristics of D_2 receptors. Chronic sulpiride treatment, however, may be able to more greatly affect the balance between D_1 and D_2 receptors. Funded by the Scottish Rite Schizophrenia Research Program.

330.5 EFFECT OF NEUROPEPTIDES ON REGULATION OF ^{3}H-SPIROPERIDOL BINDING SITES. S. M. Simasko* and G. A. Weiland (SPON: W. Schwark). Department of Pharmacology, Cornell University, Ithaca, NY, 14853.

The effect of 1 week treatment with neurotensin (NT), substance P (SP), and thyrotropin-releasing hormone (TRH) on the regulation of D_2 dopamine receptors was examined in rat central nervous system. The peptides were administered intracerebroventricularly with the use of Alzet mini-osmotic pumps. The doses administered were: NT, 4 µg/hr; SP, 3.3 µg/hr; TRH, 5 µg/hr; saline 1 µl/hr. The day after the pumps were implanted the rats were divided into two groups: one receiving haloperidol (2.5 mg/kg, i.p., twice daily) and the other a control vehicle. Haloperidol was given for 7 days. On day 8 the peptide treatment was stopped. On day 9 ^{3}H-spiroperidol binding was measured in the striatum and the nucleus accumbens.

Haloperidol treatment caused ^{3}H-spiroperidol binding to increase ≅ 30% in the striatum and ≅ 50% in the nucleus accumbens. By themselves the peptides caused no significant changes in ^{3}H-spiroperidol binding. TRH and SP administered concurrently with haloperidol was not different from haloperidol by itself. However, while the effect of NT given concurrently with haloperidol was not significant under the experiment design used (two-factor analysis of variance, $P > 0.05$), a t-test of individual results (haloperidol alone vs. haloperidol with NT) showed NT to potentiate the effect of haloperidol in both striatum and nucleus accumbens ($P < 0.05$). Additional experiments with higher doses of NT (10 µg/hr and 40 µg/hr) failed to demonstrate any effect of NT alone on regulation of ^{3}H-spiroperidol binding.

We conclude from these experiments that although SP and TRH have been shown to affect dopaminergic function in rat brain (Hanson, G.R. et al., J. Pharmacol. Exp. Ther., 218:568, 1981 and Heal, D.J. and Green, A.R., Neuropharmacology, 18: 23, 1979) the effect does not result in any long-term changes in D_2 receptor regulation. NT has been shown to modulate many dopaminergic functions in the CNS and the effect has been described as neuroleptic-like (Nemeroff, C.B., Biol. Psychiat., 15: 23, 1980). Although NT alone does not cause changes in D_2 receptor number, it does potentiate the up-regulation caused by haloperidol. (Supported by PMA Foundation, NSF BNS 82-15572, and NIH BRSG 08-57 RR05 462 E-21.)

330.6 EFFECTS OF CYCLO(LEU-GLY) ON THE DEVELOPMENT OF SUPERSENSITIVITY TO DOPAMINE AGONISTS INDUCED BY DOPAMINE AGONISTS. J.Z. Fields, J.M. Lee, K. Steece*, R.F. Ritzmann & W.Weiner* Chicago Med. Sch., U. of IL at Chicago, Univ. Miami/Parkinson's Foundation.

We have shown that cyclo(Leu-Gly)(cLG) blocks the development of supersensitivity to dopamine (DA) agonists following chronic morphine or neuroleptic administration and 6-OH-DA lesions. These treatments are thought to result in disuse supersensitivity as a consequence of "functional" DA depletion. On the other hand, it has been demonstrated that a behavioral facilitation occurs following DA agonist administration on striatally mediated DA behaviors. Therefore, the possible prophylactic effects of cLG on this agonist-induced supersensitivity were investigated. Male Swiss-Webster mice (25 to 30 g) were divided into 4 groups. Mice were injected with either cLG (8 mg/kg, s.c.) or VEHICLE-1(water followed 2 h later by either a priming dose of the DA agonist apomorphine (APO)(5 mg/kg i.p.) or VEHICLE-2(saline). 24 h later, all animals were monitored for stereotypic climbing behavior at 10 min after a challenge dose of APO(1 mg/kg,i.p.). At this time the VEH-1/APO group had a mean score of 1.13,N=15 based on a maximum score of 2.0 compared to the VEH-1/VEH-2 groups score of 0.67,N=15. However, prior treatment with cLG (APO/cLG) did not alter this agonist-induced supersensitivity (mean score 1.0,N=16). Prior treatment with cLG(cLG/VEH-2) caused a slight stimulation itself (mean score 0.90, N=9). The two types of dopaminergic supersensitivities will be discussed in relation to the possible mechanism(s) of action of cLG.

330.7 PERGOLIDE DOWN-REGULATES D-2 DOPAMINE RECEPTORS BUT FAILS TO BLOCK HALOPERIDOL INDUCED BEHAVIORAL SUPERSENSITIVITY. W.C. Koller, J.C. Curtin & J.Z. Fields, Hines V.A , Hines IL 60141 and Chicago Med Sch. North Chicago IL 60064.

We previously reported (Koller et al., Neuropharmacol., 19 (1980) 831) that pergolide appears to work only as a direct-acting D-2 dopamine receptor (DA-R) agonist in rats and in guinea pigs. Furthermore, chronic administration (4 weeks) of a dose of pergolide (0.5 mg/kg) that was below the threshold for inducing any stereotypy by itself, elicited changes that resulted in a subsensitive response to a challenge dose of apomorphine (0.2 mg/kg) that normally induces marked stereotypy. Similar exposure to other dopamine (DA) mimetics such as L-DOPA, bromocriptine or amphetamine, on the ohter hand, has just the opposite effect -induction of a behavioral supersensitivity to DA. To determine whether this apparently unique down-regulating characteristic of pergolide is generally operative, we tested whether pergolide could prevent or reverse the behavioral supersensitivity to DA that is induced by chronic haloperidol administration (0.5 mg/kg daily for 14 days). Surprisingly, pergolide (0.5 mg/kg) failed to prevent or reverse the behavioral effects of haloperidol. However, pergolide did down-regulate the D-2 DA-R binding of (3H)spiroperidol in striatal membranes from these guinea pigs by preventing the haloperidol induced increase (+ 36%) in D-2 DA-R density. Since changes in the binding of D-2 antagonists (e.g. spiroperidol) failed to correlate with changes in the behavioral response to DA, we conclude again, as we previously did (Lee et al., Life Sci. 33 (1983) 405), that the interaction of DA antagonists with striatal D-2 DA-R does not correlate with striatally mediated dopaminergic behaviors. Changes in the interaction of agonists with striatal D-2 DA-R (i.e. inhibition of (3H)spiroperidol by unlabelled DA) is a better predictor of changes in striatally mediated dopaminergic behaviors.

330.8 ISOLATION-INDUCED DECREASED DOPAMINE RECEPTOR DENSITIES REVERSED BY CHRONIC HALOPERIDOL TREATMENT. Graham Bean and Tyrone Lee. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Ontario, Canada. M5T 1R8.

An experiment was conducted in order to determine the impact of the social environment on dopamine D_2 receptor densities in the developing rat brain. Weight matched littermate male Wistar rats were removed from their maternal environment at 20 days of age and placed in isolation or in groups of three. In both conditions, animals received either haloperidol (1mg/kg/day for 40 days), vehicle (0.1M tartaric acid) or no treatment. Similar drug regimens were administered to additional rats housed in groups of three, except that two rats received haloperidol while the remaining animal received vehicle ['RRV'], or one rat received haloperidol while the remaining two animals received vehicle ['RVV']. At the end of the 40 day treatment period, animals were sacrificed and striatal tissues prepared for dopamine D_2 receptor assay using ^{3}H-spiperone and 10µM sulpiride to define specific binding. Scatchard analyses were performed to determine the density (Bmax) and affinity (Kd) of dopamine receptors. The mean ± standard error of the Bmax values are shown below:

	Grouped	Isolated	['RRV']	['RVV']
HLD	258.9±9*	211.3±9•	228.1±8•	259.6±15
VEH	199.6±11	159.7±8*	149.4±7*	199.5±8
NTR	190.2±8	146.3±6*		

There was no significant difference ($p>.05$) between Kd values for these treatment groups. HLD = Haloperidol; VEH = Vehicle; NTR = No treatment. * significantly different ($p<.05$) from no-treatment and vehicle treated grouped housed animals. • significantly different ($p<.05$) from haloperidol treated grouped housed animals.

Our present results tend to support the view that social isolation may be associated with a decrease in dopamine D_2 receptor densities, that daily handling of the animals does not attenuate this effect (as in the case of vehicle treated animals) and that chronic haloperidol treatment may reverse the decreased receptor densities to normal control values. In addition, it appears that rats are sensitive to their partner's treatment condition and that this will be reflected in the density of central dopamine D_2 receptors. Supported by NSERC & Clarke Institute Research Funds.

330.9 SHORT-TERM EFFECTS OF LOXAPINE ON RAT BRAIN DOPAMINE AND SEROTONIN RECEPTORS. Ann Goziotis* and Tyrone Lee. Psychopharmacology Unit, Clarke Inst. of Psychiatry, Toronto, Canada M5T 1R8.

Results from our previous studies (Lee and Tang, Soc. Neurosci. Abst. 9(1):33, 1983) have indicated that chronic treatment of loxapine in the rat for 4 to 10 weeks did not increase the density of brain dopamine (D_2) receptors. On the other hand, it was quite potent in reducing the number of serotonin (S_2) receptors. In order to examine if these receptors were responsive to acute or subchronic loxapine treatment in the same manner, the following investigation was carried out.

Adult male Wistar rats (175-225 gm) were divided into six treatment groups according to the duration of drug exposure: 1-day, 2-day, 3-day, 5-day, 7-day and 14-day. Animals were injected i.p. daily with either loxapine (5 mg/kg) or vehicle (1 ml of 0.1 M tartaric acid). Twenty-four hours after the last injection, they were sacrificed and brain tissue homogenates were prepared for D_2 and S_2 receptor assays. ^{3}H-Spiperone and 10^{-5} M sulpiride were used for D_2 assays on striatal tissue and ^{3}H-ketanserin and 10^{-6} M cinanserin were used for S_2 assays on frontal cortical tissues. Scatchard analyses were performed for determining receptor densities (B_{max}).

		Loxapine Treatment (days)					
		1	2	3	5	7	14
D_2	Change in B_{max} (% control)	78	65	74	82	81	117
	N	25	26	10	9	9	9
	P	<0.01	<0.001	<0.01	<0.2	<0.3	<0.4
S_2	Change in B_{max} (% control)	68	32	30	40	35	25
	N	9	9	10	10	10	10
	P	<0.05	<0.001	<0.001	<0.001	<0.001	<0.001

Short-term exposure to loxapine was shown to be very potent in significantly reducing both the D_2 and S_2 receptor densities. However, when the rats were treated for 5 or more days, the change in D_2 densities became insignificant while reduction in S_2 was maintained at around 25-40% of control. Thus loxapine appeared to cause a transient dopaminergic hyposensitivity rather than hypersensitivity on short-term exposure in the rat. The effect in serotonin receptors was also unique to this neuroleptic since haloperidol has no action on the serotonin system. (Supported by the Research Fund of the Clarke Institute of Psychiatry).

330.10 REGULATION OF [^{3}H]KETANSERIN BINDING TO SEROTONIN-2 RECEPTOR SITES IN RAT BRAIN. C.A. Stockmeier and K.J. Kellar. Dept. of Pharmacology, Georgetown Univ. Sch. of Med. Dent. Washington, DC 20007.

[^{3}H]Ketanserin selectively binds to a serotonin-2 (5-HT-2) site in brain when methysergide is used to define non-specific binding (Leysen, 1982). Here we have examined the effects of repeated administration of electroconvulsive shock (ECS), amitryptyline, reserpine, p-chlorophenylalanine (PCPA) or p-chloramphetamine (PCA) on the binding of [^{3}H]-ketanserin (≈1 nM) to homogenates of cerebral cortex and hippocampus of male Sprague-Dawley rats. Specific binding was assessed in the presence of 2 uM methysergide, LSD or 300 uM 5-HT and yielded similar results. Cold 5-HT was found to inhibit [^{3}H]ketanserin binding in the cerebral cortex and hippocampus with an IC_{50} of 0.8-3.0 uM suggesting a similar binding site in both areas. Hill slopes in both areas were shallow. Eleven days of ECS (150 mA, 0.3 sec., corneal electrodes) increased [^{3}H]ketanserin binding in frontal cortex by 33%. However, the binding in the hippocampus was unchanged. Neither the $^3IC_{50}$ nor the Hill coefficient of 5-HT in competing for [^{3}H]ketanserin sites was altered by ECS. Amitryptyline (10 mg/kg ip, 21 days) caused a 22% reduction in [^{3}H]ketanserin binding in whole cortex while binding in the hippocampus was not significantly affected. Treatment with reserpine (0.25 mg/kg, 10 days) caused a 33% increase in [^{3}H]ketanserin binding in frontal cortex, but again no change was found in the hippocampus. Neither PCPA nor PCA altered binding when measured 11 days after initiation of treatments.

In conclusion, [^{3}H]ketanserin binds to sites in the rat cerebral cortex and hippocampus. 5-HT competes for this site in both areas with an IC_{50} in the low micromolar range. Both repeated ECS and reserpine increase [^{3}H]ketanserin binding in the frontal cortex, but not the hippocampus. It appears that depleting 5-HT alone (i.e. PCPA, PCA) does not alter [^{3}H]ketanserin binding but that treatments with more wide-spread effects (i.e. reserpine, ECS) are effective in altering the sites. Thus, 5-HT-2 sites appear to be under complex control. To determine whether the ECS-induced increase and/or the tricyclic antidepressant-induced decrease in [^{3}H]ketanserin sites is dependent on intact 5-HT axons, these treatments were carried out in 5,7-dihydroxytryptamine-lesioned rats. The results of these experiments will be presented.

330.11 CENTRAL 5-HT LESIONS DO NOT PREVENT THE GREATER REDUCTION OF 5-HT_2 BINDING CAUSED BY SUBCHRONIC CHLORPROMAZINE PLUS IMIPRAMINE. T.H. Andree*, C.Y. Lee*, J.I. Koenig and H.Y. Meltzer. Dept. of Psychiatry, Univ. of Chicago Pritzker Sch. Med. Chicago, IL 60637

Previous studies in our laboratory have shown that treatment of rats with chlorpromazine (CPZ) plus imipramine (IMIP) causes a greater reduction in 5-HT_2 binding than either agent alone (Mikuni and Meltzer, Life Sci., 34:87, 1984), and may contribute to the enhanced clinical efficacy seen when psychotically depressed patients are treated with the combination of a neuroleptic plus an antidepressant.

To determine whether serotonergic neurons were required for this enhanced effect, central 5-HT lesions were produced in rats. Rats were anesthetized, pretreated with desipramine (25 mg/kg) and 30 min later given 5,7-dihydroxytryptamine (200 ug in 10 ul) into the lateral ventricle. Fourteen days later the animals were treated twice daily with saline, CPZ (5 mg/kg), IMIP (10 mg/kg) or CPZ plus IMIP for 3 days. ^{3}H-5-HT (15 nM) uptake into cortical membranes (sham controls= 582 ± 26 fmol/mg prot/5 min (n=35)) confirmed successful lesioning with an average 89% decrease in all lesioned (n=25) animals. The various drug treatments had no effect on this parameter. ^{3}H-5-HT binding (5-HT_1) to cortical membranes (control = 1.29 ± 0.08 fmol/g tissue^{-1} (at 1.0 nM 5-HT)) was unaffected by either the drug treatments or the lesioning, confirming earlier results that the 5-HT_1 binding site is not involved in the enhanced action of CPZ + IMIP.

With regards to 5-HT_2 (^{3}H-spiperone; SPIRO) binding, naive (intact) and sham-operated rats gave similar results (3.69 ± 0.20 fmol/g tissue at 0.4 nM SPIRO). CPZ alone produced a 33 and 37% reduction in binding and CPZ plus IMIP a 57 and 54% decrease in sham and lesioned animals, respectively. The ratio of binding at 1.2 and 0.4 nM SPIRO was similar in both saline and drug-treated animals indicating a decrease in B_{max} with no change in K_D. IMIP was ineffective after the 3 day treatment and the lesion itself was without efect in altering 5-HT_2 binding. From these results it is concluded that intact serotonergic nerve terminals are not required for the down-regulation of 5-HT_2 receptors produced by CPZ or by CPZ plus IMIP and confirms other reports that 5-HT neurons are not required for the down-regulation of 5-HT_2 receptors. This contrasts with the report that intact 5-HT terminals are required for the down-regulation of beta receptors by chronic tricyclic antidepressants. Whether 5-HT_2 receptors (or binding sites) are post-synaptic but not influenced by snaptic 5-HT is unclear.

330.12 5,7-DIHYDROXYTRYPTAMINE DOES NOT PREVENT THE DOWN REGULATION OF BETA-ADRENERGIC AND SEROTONIN-2 RECEPTORS AND BEHAVIORAL CHANGES IN FORCED SWIMMING TEST, INDUCED BY DESIPRAMINE.

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Recently, it was reported that the down regulation of beta-adrenergic receptor induced by desipramine(DMI) needed intact serotonin(5-HT) neurons.

We investigated the effects of 5-HT neuron lesion on DMI-induced down regulation of beta-adrenergic and S-2 receptor bindings in rat cerebral cortex. We also examined whether the destruction of 5-HT neurons prevents the behavioral "antidepressant" effect of DMI, using forced swimming described by Porsolt et al.(Eur.J.Pharmacol.1978).

Different from Porsolt who checked acute effects of drugs, we examined "antidepressant" effect after 10 days of drug administration. Of 4 drugs examined, DMI(15mg/kg) and imipramine(15mg/kg), by which the density of both 2 receptors were decreased, significantly reduced the duration of immobility(despair) of rats, and were therefore evaluated as "antidepressants". However, clomipramine(15mg/kg) and chlorpromazine(10mg/kg) were evaluated without "antidepressant" effect in the forced swimming test. The former did not alter the density of both receptors and the later decreased the density of S-2 without any change of beta-adrenergic receptor.

Lesion of 5-HT neurons (i.c.v. administration of 5,7-dihydroxytryptamine) decreased 5-HT content in frontal cortex by about 80% as compared with that of sham-lesioned rats without significant changes of NE and DA contents determined by HPLC. Such lesion did not alter the density not only of beta-adrenergic but also of S-2 receptors. Moreover, DMI-induced down regulation of beta-adrenergic and S-2 receptors was not affected by 5-HT neuron destruction (percent decrease of receptor number induced by DMI were 29.8% and 25.0% for beta-adrenergic, and 29.3% and 33.0% for S-2 receptors in sham-lesioned and lesioned rats respectively). In accordance with these findings,"antidepressant" effect of DMI in forced swimming test was not influenced by lesion of 5-HT neurons.

330.13 EFFECTS OF SINGLE INJECTION OF CHLORPROMAZINE AND/OR YOHINBIN ON SEROTONIN-2 RECEPTOR BINDING IN RAT CEREBRAL CORTEX. Masahiko Mikuni*, Shigehiro Matsubara*, Itaru Yamashita* and Herbert Y. Meltzer[2] (SPON. John Metz, Ph.D.), Dept. of Psychiat. Hokkaido Univ. Schl. of Med. Sapporo, Hokkaido, 060, Japan and [2]Dept. of Psychiat. Univ. of Chicago Pritzker Schl. of Med. Chicago, IL 60637

We reported previously that chronic administration of some neuroleptics reduced the density of serotonin-2(S-2) receptor binding sites as well as various antidepressants. Main interest in this field is why S-2 antagonists reduce the density of S-2 receptor binding sites, whereas chronic treatment with D-2 antagonists increase the number of D-2 receptor binding sites.

We investigated the time course of S-2 receptor number alteration induced by single injection with 10mg/kg of chlorpromazine(CPZ) and/or yohinbin(YOH), or mianserin(MSN). Animals were sacrificed 2, 6, and 24h after these single injections. The maximal reduction in S-2 binding occured 2h after the injection with CPZ alone, CPZ plus YOH, or MSN by 80%, 80%, 60%, respectively compared with saline controls. This reduction in S-2 receptor number by CPZ was not influenced by preincubation of homogenized tissues with Tris buffer for 20min at 37°C before centrifugation. By 24h the density of S-2 receptor binding sites in CPZ treatment group almost retured to control values, while the number of S-2 receptors in CPZ plus YOH, and MSN alone groups were still about 40% lower than controls. These results suggest the acute effect of MSN on S-2 receptors, reported by Blackshear et al.(J.P.E.T.,1982), due to the interaction with alpha-2-adrenergic antagonistic property of MSN.

We also examined the effect of various doses of CPZ on S-2 receptor density at 2h after the injection. The density of S-2 binding sites significantly decreased at 2, 5, and 10mg/kg of CPZ by 30%, 60%, 80%, respectively.

In addition, we investigated whether 5,7-dihydroxytryptamine(DHT) pretreatment(i.c.v.) prevent this reduction in S-2 receptor number induced by 10mg/kg of CPZ, at 2h after the injection. The 80% reduction in S-2 receptor number in DHT pretreated group was observed as well as in sham-lesioned group.

These results indicate the possibility of direct effect of CPZ on postsynaptic S-2 receptors, and of the interaction with alpha-2-adrenergic receptors to produce the down regulation of S-2 receptors.

330.14 INFLUENCE ON [^{3}H]-5-HYDROXYTRYPTAMINE BINDING SITE DEVELOPMENT IN CHICK EMBRYO BY SEROTONERGIC COMPOUNDS. J.S. Soblosky and I. Jeng*. Neurochem. Unit, Missouri Inst. Psychiatry, Dept. Biochem., Univ. Missouri-Columbia, Sch. Med., St. Louis, MO 63139

Saturable and specific binding sites for [^{3}H] 5-hydroxytryptamine ([^{3}H] 5-HT) characterized by a K_D of 3.5-4.5 nM were detected in the chick embryo brain and were shown to develop linerally as a function of age, weight and protein content. Saturation and displacement studies using unlabeled 5-hydroxytryptamine as the displacing ligand suggested a single population of binding sites. Displacement studies using 5-methoxytryptamine, lysergic acid diethylamide (LSD) 2-bromo-lysergic acid diethylamide (BOL), methysergide and spiperone as competing ligands suggested the existence of subclasses of [^{3}H]5-HT binding sites because the Hill coefficients were less than unity. When compared with the [^{3}H]5-HT binding sites in the rat forebrain (5-HT$_1$) the IC_{50} values of the drugs are similar. However, differences in the Hill coefficients for lysergic acid diethylamide and methysergide suggested that the [^{3}H]5-HT binding sites in the chick embryo brain may be more similar to those found in rat spinal cord than rat forebrain.

To study [^{3}H]5-HT binding site regulation and development various serotonergic compounds were injected into the chorioallantoic fluid of the eggs at different times during embryonic development. Multiple pretreatments with d,l-5-hydroxytryptophan, 5-hydroxytryptamine or 2-bromo-lysergic acid diethylamide were found to have no effects on either the affinity (K_D) or number (B_{max}) of specific [^{3}H]5-HT binding sites. Multiple pretreatments with para-chlorophenylalanine were found to increase specific [^{3}H]5-HT binding 23% ($p < .01$) while multiple pretreatments with lysergic acid diethylamide were found to decrease specific binding 45% ($p < .01$). Neither of these pretreatments affected K_D values which indicated that only the number of specific [^{3}H]5-HT binding sites were altered. Evidence was presented suggesting that these effects were probably not due to the amounts of endogenous 5-hydroxytryptamine or lysergic acid diethylamide remaining in the tissue preparation. The overall evidence indicated that the chick embryo brain may have a functioning serotonergic system and that the chick embryo may be an ideal system for the study of [^{3}H]5-HT binding site regulation and development.

330.15 AUTORADIOGRAPHIC LOCALIZATION OF ALTERATIONS IN [^{3}H]-IMIPRAMINE, [^{3}H]-SEROTONIN AND [^{3}H]-KETANSERIN BINDING SITES IN THE RAT CNS AFTER CHRONIC IMIPRAMINE TREATMENT. D.R. Gehlert* and J.K. Wamsley (SPON: J. Baringer). Dept. of Psych. and Pharm., Univ. Utah, Salt Lake City, UT 84132.

Tricyclic antidepressants (TCA) produce rapid increases in the synaptic availability of monoamines. Imipramine (IMI) produces this effect principally by an inhibition of the neuronal reuptake of serotonin (5HT). Since the clinical onset of action requires several weeks, this acute effect does not appear to be directly responsible for antidepressant actions. A number of investigators have reported a selective "down-regulation" of the 5HT-2 receptor subtype occurs in rats after chronic IMI administration. This reduction in binding takes place with a time course similar to that required for the clinical remission of symptoms of depression seen in human patients undergoing TCA therapy. Recently, specific binding sites for [^{3}H]-IMI have been localized to serotonergic nerve terminals in the rat brain. Intrinsic to a functional role for these binding sites is that the reduction in 5HT-2 binding should occur in areas of the brain containing a high density of IMI binding sites. Therefore, receptor autoradiography has been employed to define the specific brain areas exhibiting alterations in serotonergic binding sites following chronic IMI treatment.

Rats were administered 10 mg/Kg IMI twice daily for 3 weeks. Sections of brain tissue were then labeled with [^{3}H]-IMI, [^{3}H]-5HT or [^{3}H]-ketanserin. Autoradiograms were generated by apposition of the labeled tissue sections to LKB Ultrofilm. After development, the grain densities produced by the specifically bound ligands were analyzed by computer assisted microdensitometry.

Using these procedures, chronic IMI administration was found to induce a marked reduction in [^{3}H]-ketanserin binding to 5HT-2 receptors in several brain nuclei including the anterior medial and anterior ventral thalamus, substantia nigra, olfactory tubercle and the lateral septum. Autoradiograms generated by serial tissue sections labeled with [^{3}H]-IMI displayed an increased grain density associated with the caudate-putamen, lateral septum and olfactory tubercle. The reductions in 5HT-2 receptor binding were confined at areas containing a high density of IMI binding sites. These data suggest a functional role for the IMI binding site in causing the "down-regulation" of 5HT-2 receptors which occurs following IMI administration.

331.1 BRAIN STEM DYSFUNCTION AFTER SEVERE HEAD TRAUMA EVIDENCED BY SOMATOSENSORY AND AUDITORY EVOKED POTENTIALS (EP's). L. Silbert*, P. Newlon* and D. Becker. Div. of Neurosurg., Medical College of Virginia, Richmond, VA 23298-0001

Although coma, itself, is commonly considered to be due to brain stem (BS) dysfunction, an objective account of the incidence and implication of BS dysfunction in comatose head-injured patients is not available. We have therefore analyzed the conduction values (CV) obtained in 59 of these patients who demonstrated intact somatosensory (SEL) and auditory (BAEP) early latency EP's, as they relate to outcome. Patients with grossly abnormal or absent EP's were not included, nor were those who did not receive both tests.

CV's were obtained from interpeak latencies of waves I-V in the BAEP and N13-P17 (recorded at C7) in the SEL. CV's were considered abnormal if they were - 2 SD of the normative mean.

Of 59 patients, 50% had normal SEL and BAEP CV's, 20% had BAEP abnormalities only, 15% had SEL abnormalities only and 15% had delayed conduction in both modalities. Outcomes of these groups are shown below:

Abnormality	n	Outcome (%) Good/ Moderate	Severe/Vegetative	Dead
None	29	55	17	28
BAEP only	12	58	8	33
SEL only	9	44	44	11
Both modalities	9	33	11	56

These results suggest that even though the brain stem may be grossly intact, dysfunction as evidenced by EP conduction delays is rather common, albeit transient in some cases. Precise correlations between the EP indices of dysfunction and clinical measures were not found, leaving the neurophysiologic basis of the abnormalities in question.

The EP abnormalities do not appear to be particularly useful, by themselves, in clearly discriminating outcome, although delays in both modalities do point to a poor prognosis in general. The finding that SEL's and BAEP's are normal after injury in half of the patients does not rule out dysfunction in areas not tapped by these modalities. However, the poor outcomes seen in this group were often associated with secondary insult and/or diffuse hemispheric damage as seen in long latency visual and somatosensory EP's. This work was supported in part by NIH Grant NS 12587.

331.2 EFFECTS OF PERFLUOROCARBON EMULSION TRANSFUSION, HYPERVOLEMIC HEMODILUTION AND ALTERATIONS OF PROSTAGLANDIN SYNTHESIS ON POST-TRAUMATIC RECOVERY OF SPINAL CORD CONDUCTION IN RATS. H. F. Martin, J. G. Blackburn, S. Katz and M. Rowland*. Dept. Of Physiology, Medical University of S. C., Charleston, S. C. 29425.

Rats were anesthetized with pentobarbital (25 mg/kg, I.P.) and ketamine (83 mg/kg, I.M.) for surgical preparation then maintained by supplemental ketamine alone. Somatosensory evoked potentials (SEP's) were recorded bilaterally from stimulation of sciatic nerves before and after calibrated spinal cord trauma was produced by a weight-drop device with contact surface area of 5.4 mm^2. SEP's were monitored at regular intervals during 4-6 hours of acute recovery. At 4-8 g-cm trauma, SEP's fully recovered following a loss immediately after injury. With 10-16 g-cm trauma, slow recovery occurred over a 2-4 hour period, frequently terminated by a secondary loss at 3 1/2-5 hours. With trauma of 20-24 g-cm, most animals had no recovery of SEP's. Thus 24 g-cm trauma was used as the trial level since it is just above the level of variable recovery in the untreated animal.

Subsequent sets of rats were treated with an agent to alter a chosen hemodynamic factor and SEP recovery assessed after 24 g-cm trauma. In order to reduce the contribution of the formed elements of the blood to vascular reactions after trauma, animals were transfused with perfluorocarbon emulsion (Oxypherol, Alpha Therapeutics) to Hct. $<10\%$. This resulted in most animals having partial recovery of SEP's. Hypervolemic hemodilution with 4cc of 10% Dextran-40 resulted in marked improvement in recovery in most animals. If animals were pre-treated with the cyclo-oxygenase inhibitor Ibuprofen (Upjohn, 15 mg/kg), most had partial recovery followed by secondary loss of SEP's by 4-5 hours. If animals were pre-treated with the thromboxane synthetase inhibitor Dazoxiben (Pfizer, 30 mg/kg), most had a maintained substantial recovery.

This preparation therefore appears to be a good model to assess transient changes in conduction following spinal cord trauma. These results suggest that alterations in hemodynamic factors play a significant role in recovery of spinal cord conduction and that prostaglandins, especially thromboxane, may mediate part of the response to trauma. (Supported by NINCDS grant # 2P01-NS11066-10)

331.3 THE EFFECTS OF SPINAL CORD INJURY ON RECORDINGS OF A MOTOR EVOKED POTENTIAL IN THE SPINAL CORD AND PERIPHERAL NERVES. M. McCaffrey, W. J. Levy, T. Spagnolia, D. H. York. Division of Neurosurgery, University of Missouri School of Medicine, Columbia, Missouri 65212.

In order to develop an experimental and clinical tool for evaluation of central nervous system damage, we have been monitoring the corticospinal evoked potential created by stimulation of the motor cortex, either directly or transcranially through the scalp (Levy, York, McCaffrey and Tanzer, NEUROSURGERY, August 1984). Stimulation of motor cortex produces a descending signal with contralateral limb activation which follows traditional descriptions of motor cortex pyramidal tract activation. Adult cats are anesthetized with Ketamine and Rompun, maintained on a respirator, surgical exposures are done of the spinal cord in the mid-thoracic, low thoracic and lumbar area. The peripheral nerves, sciatic and radial, are also exposed for monitoring. Recording electrodes are placed at these sites and a Cadwell 7400 signal averager is used, with the stimulator being either the Cadwell stimulator or a Grass S88 through an SIU 7 and CCU1 stimulus isolation unit. Currents of 10 to 20 milliamperes are delivered transcranially between a scalp electrode over the cat motor cortex and a second electrode up against the hard palate. Three types of spinal cord manipulations were carried out for evaluation. In one a balloon inserted into the epidural space at the high thoracic area was progressively inflated and the somatosensory and motor evoked potentials were monitored. It was observed that both potentials would first shift their latency and then decrease their amplitude as the pressure increased. The motor evoked potential in some cases demonstrated a shift in amplitude before the somatosensory evoked potential, recovered more slowly. However, at a point where the balloon compression caused complete loss of the somatosensory evoked potential, a small but delayed motor evoked potential was present. Secondy, a weight drop technique was carried out on the spinal cord. The motor evoked potential would diminish with the impact and then return over a period of 5 to 15 minutes. In some cases, a large first wave appeared with loss of later waves, previously described as an injury potential. Thirdly, the spinal cord was cut using either a CO_2 laser or a section of razor blade. The peripheral nerve signal of the motor evoked potential depended entirely upon continuity of the lateral corticospinal tract. These studies are indicative that the motor evoked potential can be used as a monitoring tool in experimental work for studying central nervous system injury. It is also potentially valuable as a clinical tool.

331.4 INTRACORTICAL LAMINAR RESPONSES TO MONOCULAR FLASH STIMULI M.A. Kraut*, J.C. Arezzo, and H.G. Vaughan, Jr. Dept. of Neuroscience and Neurology, Albert Einstein Coll. of Med. Bronx, N.Y. 10461

In a continuing effort to define the generators of the surface recorded VEP, we have compared the intracortical distribution of flash visual evoked potentials (VEP), multiple unit activity (MUA), and current source density (CSD) in the monkey striate cortex under ipsilateral and contralateral monocular conditions. Data were collected from 40 depth passes in 4 unanesthetized monkeys (M. fascicularis) using a 16 channel, multicontact electrode, with contact impedances of approximately 300 kOhms and spacings of 75 um.

The MUA within lamina IV was the measure most clearly differentiated with monocular stimulation. At several sites, unit firing increased approximately 100%, as compared to spontaneous rates, following stimulation of one eye, while showing little or no increase in activity following stimulation of the opposite eye. The increased MUA is present from 18 to 50-60 msec following stimulation. An approximately equal number of contralateral and ipsilateral dominant sites were encountered. At each site the binocular MUA resembled the dominant monocular activity. Current sources and sinks within and adjacent to lamina IV contain oscillations consistent in frequency and phase with those seen in the MUA. The most consistent VEP and CSD changes associated with monocular stimulation were asymmetries in amplitude of the N40 and N55 components that are associated with neuronal activation in lamina IVCb (Kraut et al., Neurosci. Abstr. 9, 1983).

Our findings show clear horizontal ocular dominance regions within striate lamina IV in the monkey to diffuse flash. These data are in conformity with the segregation of monocular cortical projections and responses of single units within lamina IV. The combination of MUA and VEP recordings provides a physiologic profile of laminar activity that is capable of resolving the response of striate cortex vertically within the sublaminae of the thalamo-recipient layer and horizontally to at least the dimensions of ocular dominance regions.

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331.5 EVOKED POTENTIAL PATTERNS IN DIFFERENT AREAS OF THE AUDITORY CORTEX OF THE BEHAVING CAT.
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The regional differences in the evoked potential /EP/ components of different auditory cortical areas were studied with chronically implanted multielectrodes which allowed the simultaneous recording of the field potentials from the surface and from six consecutive depths of the cortex /Karmos et al. Physiol.& Behav.1982,29:567/. The stimuli were clicks or electrical impulses given to the medial geniculate body /MGB/. Three dimensional computer graphics were used to visualize the evoked intracortical potential fields.

After habituation the EPs recorded from a given cortical point were remarkably stable in identical behavioral states, while EPs of different cortical areas had widely different waveform patterns. Changes in the animals' behavior, e.g. in wakefulness-sleep cycle and during aversive conditioning were accompanied by conspicuous waveform alterations. In the alert animal the most characteristic EP components recorded from the surface of the A.I. area were the early P10, N15 and the middle latency P50 and N70 waves. EPs in A.II. area consisted of early P11, P20 and middle latency N50 and P80 components. In drowsy and sleeping animal both the amplitude and the latency of the middle latency components increased in the A.I. area, while in the A.II. area latency increase of the middle latency components was accompanied by gradual amplitude decrease. The early components displayed only minor amplitude fluctuations. Only the first positive component showed phase reversal in the depth of the cortex, the potential fields of the longer latency components had more homogeneous distribution.

Electrical stimulation of the MGB induced EP patterns in the auditory cortex similar to those elicited by click stimuli. The lesion of the lateral part of the MGB abolished not only the early but all the components in the A.I. area which questions the role of the time locked non-specific reticulo-thalamic input in the genesis of the longer latency cortical EP components.

331.6 ENHANCEMENT OF MIDDLE LATENCY AUDITORY EVOKED POTENTIALS IN NORMAL HUMAN AGING
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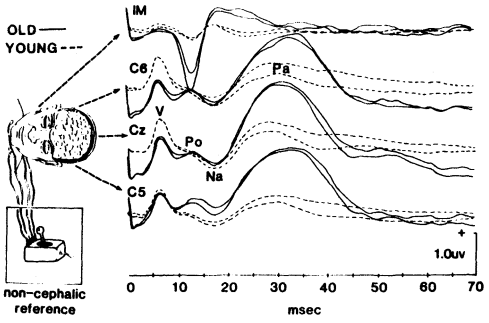
We compared middle latency auditory evoked potentials (MAEPs) in populations of young (age 18-35 yrs) and elderly (ages 60-70 yrs) subjects. MAEPs were elicited by monaural and binaural clicks presented at rapid rates (13/sec), and were recorded from the vertex, over temporal sites, and from the mastoid processes. In six different conditions stimuli were presented to the left ear, binaurally, or to the right ear at different intensities (50 or 60 dB SL).

In all conditions the elderly showed a striking enhancement (more than 200%) in the amplitude of the Pa component. These differences are illustrated below in the grand mean ERPs from one condition (binaural stimulation at 50 dB above threshold).

The effects of the ear of stimulus delivery on MAEP distributions and the effects of binaural interaction will also be discussed.



331.7 MIDDLE AND LONG-LATENCY AUDITORY EVOKED POTENTIALS IN PATIENTS WITH BITEMPORAL LESIONS
David L. Woods, C. C. Clayworth*, R. T. Knight*, and G. Simpson*. Clinical Neurophysiology Laboratory, Dept. of Neurology, UC Davis, VA Medical Center, Martinez, Ca. 94553

We recorded middle latency auditory evoked potentials (MAEPs, latency range 10-70 msec) and long-latency AEPs (N100 and P200) in five patients (ages 39 - 82 yrs) with bitemporal lesions localized by CT scan. The patients showed varying degrees of damage of primary auditory cortex and auditory associations areas, and auditory symptoms ranging from receptive aphasias to total cortical deafness.

MAEP recording revealed three different patterns of results: 1) Two patients (A.B. and V. B.) showed normal MAEPs with symmetrical distributions and comparable amplitudes following stimulation of either ear. 2) Two patients (H. H. and C.B.) with more severe involvement of the left hemisphere showed reduced but symmetrical MAEPs following right ear stimulation, and normal MAEPs following left ear stimulation. 3) One patient (L.W.) with involvement of mesial parietal areas of the left hemisphere showed an asymmetrical reduction in MAEP amplitudes on that side. In addition, MAEPs were smaller following right than left ear stimulation.

Long-latency AEPs were recorded in four of the patients. Again three patterns were observed: 1) In two patients (A.B. and H. H.) with lesions restricted to the temporal lobes, N100s had normal amplitudes and distributions. 2) In one patient (V. B.), with damage extending into the inferior parietal lobe, the N100 was reduced bilaterally. 3). In the remaining patient (L. W.) with involvement of mesial parietal areas on the left, N100 amplitudes were small and reduced over the affected hemisphere.

MAEPs and long-latency AEPs were dissociated by brain lesions in two patients. In one patient (V. B.) MAEPs were intact but the N100 was reduced, while in another (H. H.) ear asymmetries were found in the MAEPs, but the N100 was normal.

The discussion of these results will center on the neuroanatomical generators of middle and long-latency AEPs.

331.8 MIDLATENCY AUDITORY EVOKED RESPONSES: DIFFERENTIAL EFFECTS OF SLEEP IN THE HUMAN.
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In normal adult humans, auditory stimuli evoke a series of short latency (10 ms) evoked responses. These auditory brainstem responses (ABRs) are now known to be generated by the auditory relay nuclei of the brainstem due, in part, to systematic study of the ABRs in animal models, e.g., the cat. Subsequent to the ABRs, middle latency responses occur in human and also in the cat model. Of particular interest in the cat is a 20-25 ms positive potential (wave A) which appears to reflect a generator system extending from the mesencephalic reticular formation upward to the intralaminar thalamus. Insofar as this middle latency potential diminishes and disappears during Stage 3 and 4 sleep in the cat (Chen, et al., Neurosci. Abst. 10:1984), a similar study of middle latency responses during sleep was undertaken on human subjects. Middle latency potentials were recorded from a group of normal adult subjects during periods of wakefulness and sleep in response to 1/sec clicks (0.1 ms, 50 db HL). Evoked potentials were recorded from the vertex (Cz) and bipolar eye and chin electrodes were used to monitor eye movement and muscle activity. For each block of 500 stimuli an averaged evoked response was generated. Sleep stages were assessed through inspection of on-going EEG records and later confirmed through spectral analysis.

A series of prominent middle latency components were observed in each waking subject: 1) a positivity 30-40 ms following stimulus onset, 2) a negativity at 40-60 ms, 3) a second positivity at 60-75 ms, and 4) a second negativity occurring at 75-90 ms. These potentials correspond to those described by Picton et al. (EEG 36: 179, 1974) as Pa, Nb, Pl, and Nl, respectively. During Stage 3 and 4 sleep the amplitude of the first positivity (Pa) increased. The amplitude of the second pocitivity (Pl) decreased or disappeared entirely, so that the precedant and subsequent negativities appeared fused. These findings suggest that certain middle latency components are sensitive to changes in arousal. The similarity between the human 60-75 ms positivity, "Pl", and the cat 20-25 ms positivity, "wave A", both in general morphology and behavior during waking-sleep stages, suggests that these waves may share a common generator substrate.
(Supported by USPHS HD-05958 and HD-04612).

331.9 MIDLATENCY AUDITORY EVOKED RESPONSES: DIFFERENTIAL EFFECTS OF SLEEP IN THE CAT. B.M. Chen*, J. Buchwald, C. Shipley*, Brain Res. Inst., Ment. Ret. Res. Ctr., Dept. Physiol., UCLA Med. Ctr., Los Angeles, CA 90024.

In previous work we have shown that a sequence of auditory evoked potentials can be recorded from the vertex of the awake cat in response to repetitive click stimulation. The auditory brainstem response (ABR), originating within the first 10 ms from the auditory brainstem relay nuclei, is followed by middle and long latency potentials. A positive potential of 10-15 ms latency appears to be generated by primary auditory cortex, e.g., the response occurs at the latency of the primary cortical response, shows similar recovery cycle characteristics and disappears with bilateral ablation of auditory cortex. The subsequent positive potential with a 20-25 ms latency (wave A) reflects a different generator. As indicated by correlative measures of surface and depth field potential and single unit latencies, recovery cycles, and lesion studies, this generator system extends from the dorsomedial midbrain reticular formation to the centre median and centralis lateralis nuclei of the thalamus. Wave A disappears under pentobarbital anesthesia whereas the precedent ABRs and cortical potential do not. We hypothesized that wave A, but not the earlier potentials, would also disappear during Stage 3 and 4 sleep, periods of decreased activity in the reticular arousal system. Vertex recordings were carried out on restrained adult cats with the head held in a constant position for free-field sound stimulation. During recording sessions click stimuli were presented at 1/sec and 50 trial blocks were averaged on a DEC 11/23 computer. Following 6 awake recording sessions, which sampled responses over 30 to 40 min, the cat was sleep deprived over a 2 day period. Subsequent evoked response recordings were again carried out over a 6 to 8 hour period, during which the cat was allowed to sleep ad lib. Concurrent EEG activity was tape recorded for later off-line spectral analysis. During Stage 3 and 4 sleep, indicated by neck EMG, eye movement and EEG measures, wave A and its subsequent negativity diminished or disappeared completely. A middle latency component in the human, "P1", also disappears during Stage 3 and 4 sleep (Erwin, et al, Neurosci. Abst. 10: 1984). These data suggest that wave A in the cat, (and possibly "P1" in the human), provides an electrophysiological window into the reticular arousal system. (Supported by USPHS HD-05958, and HD-04612).

331.10 CAT P300 INDEPENDENT OF PRIMARY AUDITORY CORTEX. J. Harrison and J. Buchwald. Brain Res. Inst., Ment. Ret. Res. Ctr., Dept. Physiol., UCLA Med. Ctr., Los Angeles, CA 90024.

The human P300 potential is a response to unexpected stimuli presented randomly within the context of expected stimuli. It appears to measure stimulus discrimination, sequential information processing, and short-term memory and has become increasingly interesting to neurologists, psychiatrists and other clinicians, since it is abnormal in some brain diseases, e.g., Alzheimer's. The diagnostic utility of the P300, and the cognitive functions it reflects, make the question of its generator substrate particularly compelling. We have reported (Buchwald, J. and Squires, N. In: Conditioning, Plenum, N.Y., (C. Woody, Ed.) 503, 1982; J. Harrison and J. Buchwald Neurosci. Abst. 9: 1196, 1983) an endogenous response in the awake cat which occurs with a typical P300 protocol, is task related, and shows a maximum positivity in the 200-500 msec latency range which, with principal component analysis (PCA), shows significantly different factor scores for rare versus frequent stimuli, all characteristics of the human P300.

The present study assessed the role of the primary auditory cortex as a possible generator of the auditory P300. In daily recording sessions, stimuli were randomly ordered with a loud click frequent (P=.80) and soft click rare (P=.15) during two 500-trial blocks. Stimulus probability was counter-balanced in two other 500-trial blocks. A 4KHz tone followed by eyelid shock also occurred rarely (P=.05) and provided an eyelid conditioned response which focused the cat's attention. PCA and paired T-tests were used to compare responses to the rare and frequent click stimuli across 10 pre-operative control sessions. Aseptic surgery was then carried out under pentobarbital anesthesia and the mid-ectosylvian gyrus (primary auditory cortex) was aspirated bilaterally. Following a 2 day recovery period, responses to rare and frequent click stimuli were again recorded across 10 sessions. Paired T-test comparisons of the rare vs. frequent loud click responses showed a significant difference between data points in the 200-500 msec latency range before surgery. This significant difference was still present after surgery. Subsequent histology demonstrated complete bilateral ablation of the mid-ectosylvian gyrus, with adjacent cortex spared. These results suggest that primary auditory cortex does not contribute significantly to auditory P300 generation. (Supported by USPHS HD-05958, AG-04088, and HD-04612).

331.11 **AUDITORY BRAINSTEM RESPONSES (ABR'S) IN NORMAL AND PAROXYSMAL WHITE LEGHORN CHICKS.** **M.M. Beck and T.A. Jones, Depts. of Animal Science, University of Nebraska-Lincoln, and Oral Biology, University of Nebraska Medical Center, Lincoln, NE 68583**

The paroxysmal (px) chick is a mutant which appears normal at hatching and during the subsequent week. By approximately 9d posthatching, various symptoms develop, of which the most obvious are depressed food intake (anorexia) and audiogenic seizures (Cole, R.K., J. Heredity 52:47-52, 1961; Kuenzel, W.J. and J.B. Rubenstein, Exp. Zool. 187:63-70, 1974). Histological evidence suggests that central auditory and vestibular nuclei and fiber tracts begin to degenerate prior to seizure onset. This degeneration, which affects central and peripheral components of both systems, becomes increasingly severe over time although auditory stimulation continues to elicit seizures (Beck, M.M., et al., Brain Res. 260:11-20, 1983). The observed anatomical abnormalities prompted the present study of auditory function in these birds. Chicks used were approximately 3.5-week-old px (5) and non-px (3) siblings. Clicks (.06 ms duration) of approximately 40, 50, or 60 dBHL were delivered monaurally through a 2.5" plastic tube. In each chick, stainless steel wire electrodes were sutured into the skin overlying the vertex and base of the skull. EEG signals were amplified (10K), filtered (LF300, HF10K), and led to a signal averager. For recordings, 512 responses were averaged (10/sec stimulation rate) to produce ABR's. Seven major positive peaks were identified in the normal birds. In all birds, hypothermia produced progressive delays in peak latencies, suggesting (but not proving) that the major peaks were organized in a serial fashion, where each successive peak may represent activity in more rostral structures. Major response differences between px and normals occurred for peaks later than 3A. Substantial decreases in the amplitude of wave 4 occurred in all px chicks. Latencies were substantially longer for all peaks in px chicks when compared to the normal counterparts at equivalent stimulus intensities, a phenomenon perhaps due in part to lower px body temperatures.

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331.12 PRINCIPAL COMPONENT ANALYSES OF SENSORY EVOKED POTENTIALS IN THE DENTATE GYRUS OF THE RAT. R.E. Hampson*, T.C. Foster*, E.P. Christian, M.O. West and S.A. Deadwyler, Dept. of Physiology & Pharmacology, Bowman Gray Sch. Med., Winston-Salem, NC 27103

In previous studies we determined that the auditory evoked potential recorded from the outer molecular layer of the dentate gyrus (OM AEP) contains two prominent negative waves (N1 and N2) which are differentially affected by various behavioral treatments as well as by lesions of different afferent fiber systems to this region (Deadwyler, et al., Science, 211:1181, 1981). In the present study, principal component analysis was utilized to characterize the relationship between the waveforms of the OM AEP and the conditions under which identified components are differentially altered during performance of a two-tone auditory discrimination task.

Principal component analysis yielded five basic waveforms which accounted for over 90% of the total variance of the OM AEP waveform. Three of the basic waveforms included components corresponding to the N1 wave. N2 waves were identified in all five basic waveforms, appearing large in those with N1 contributions. A late positive wave (P2) appeared only in basic waveforms with N1 waves. Basic waveforms which included an N1 wave accounted for 42% of the variance. Basic waveforms which did not include an N1, but retained an N2 wave accounted for the remainder of the N1 and N2-dependent (OM AEP) waveform variance.

A second analysis extracted principal components from OM AEPs which were sorted and averaged on the basis of individual trial sequences (West et al., Neurosci. Lett., 28:319, 1982). For four different animals, five basic waveforms exhibited combinations of N1, N2 and P2 waves, accounting for over 80% of the variance. Three of these basic waveforms showed consistent N1 and N2 amplitude changes across 64 possible combinations of 5-trial sequences. 28% of the variance was associated with basic waveforms showing N2 amplitude greater than N1 amplitude. 21% of the variance was associated with basic waveforms exhibiting N1 amplitude greater than N2 amplitude. This differentiation corresponded to trial sequences which could be segregated with respect to overall probability of a positive (N2) or negative (N1) trial. Data from each animal scored by the weighting coefficients showed significant sequence effects ($.001 \leq p \leq .05$ by ANOVA) over different types of sequence. These results confirm our assumption as to the independence of the N1 and N2 portions of the OM AEP waveform.

331.13 EFFECTS OF OPERANTLY CONDITIONING CORTICAL SOMATOSENSORY EVOKED POTENTIAL (SEP) AMPLITUDE ON CONTINUOUS NON-TIME-LOCKED MOVEMENT AND REFLEX MOVEMENT IN THE RAT. J.P. Rosenfeld and R. Dowman, Dept. of Psychology, Northwestern University, Evanston, IL 60201 and NYS Dept. of Health, Ctr. for Labs and Research, Albany, NY 12201

Continuous non-timelocked movements (unrelated to the evoking stimulus) have been shown to decrease SEP amplitude. (Hazemann et al, EEG Cl. Neuro 39:247, 1975). We were interested in determining whether such movement could be mediating operantly conditioned changes in SEP amplitude. We also measured reflexive movements of the head and upper body produced by the evoking stimulus (which was innocuous stimulation of the spinal trigeminal tract) during training.

Rats (6) were rewarded with electrical stimulation of the medial forebrain bundle for making the amplitude of a 30 msec surface positive component (P2) of the SEP larger than (uptrain) or smaller than (downtrain) the predetermined mean of that component. Movement was monitored by a movement transducer (2 phonograph cartridges connected in series) mounted (perpendicular to one another) on the animal's recording cable near its head. We have used and validated this device before and have found it to be a reliable and valid measure of relative amounts of movement (Hetzler et al, Physiol. Beh. 21:1047, 1978). Continuous non-timelocked movement was analyzed by rectifying and summing the movement transducer output over a 600 msec epoch occurring before the delivery of the evoking stimulus. Likewise, movement transducer output occurring during the 200 msec epoch following the evoking stimulus was rectified and summed. These two measures provided separate estimates of the relative amounts of movement occurring during training. Reflex movement was analyzed by averaging the movement transducer output in exactly the same fashion as the SEP.

All animals successfully conditoned (i.e. uptrained P2 amplitude was larger than downtrained P2 amplitude). There was no difference in continuous non-timelocked movement between uptraining and downtraining (P>.10). This result demonstrates that continuous non-timelocked movement is not a necessary mediator of SEP conditioning. Reflex amplitude, however, did change with training. Reflex amplitude was larger in uptraining than in downtraining ($p<.05$). This result demonstrates that in addition to altering nociceptive threshold (Dowman et al, Brain Res. 269:111, 1983) operantly conditioning cortical SEP amplitude can affect innocuous subcortical reflex activity.

331.14 Spontaneous membrane potential fluctuations in hippocampal CA1 cells in urethane-anesthetized rats. L. S. Leung and C.Y. Yim. Dept. of Psychology , Univ. of Western Ontario, London, Canada N6A5C2 and Dept. of Anaesthesia Research, McGill Univ., Montreal, Canada H3G1Y6.

Intracellular recordings were obtained from more than 40 hippocampal CA1 cells in urethane-anesthetized (1.2 g/kg i.p.) rats, using potassium acetate or chloride micropipets. Inhibitory postsynaptic potentials (IPSPs) were seen in many cells following fimbrial or alvear stimulation (at 0.1-0.2 Hz). In addition, many cells showed spontaneous fluctuations of the membrane potentials, of which the oscillation in the theta frequencies (3-5 Hz) could reach 10 mV in amplitude. The extracellular theta in the pyramidal cell layer seldom exceeded 0.5 mV. An extracellular electrode was placed in the contralateral hippocampus to record spontaneous EEG. The intra- and extracellular signals were analyzed by fast Fourier transform and autopower, cross-coherence and cross-phase spectra (0.5-100 Hz) were plotted. With acetate/chloride ion diffusion or by means of passage of hyperpolarizing currents(1-10 nA), the evoked IPSP was reversed from a hyperpolarizing to a depolarizing direction. Concomitant with IPSP reversal, the phase of theta between intra- and extracellular signals also changed. Eight CA1 cells showed a 180 degrees shift in this phase, while 3 others showed a 45-80 degrees shift. Antidromic action potentials following alvear stimulation were found in 5 out of 8 cells in the former group. The amplitude of the intracellular fluctuations in the theta or non-theta (0.5-100 Hz) range showed a direct correlation with the amplitude of the IPSP measured at about 10 msec following alvear stimulation. When irregular slow activities predominated (instead of theta), both intracellular and extracellular records gave similar power spectra except coherence between the records was low at all frequencies. Glial cells did not have large transmembrane potential fluctuations.

This study reveals that physiologically identified CA1 projection (probably pyramidal) cells possess transmembrane oscillations in the theta frequency, which behave like rhythmic IPSPs. It also shows that fluctuations in the membrane potentials at theta or non-theta frequencies are likely responsible for the generation of the extracellular EEG. Direct feedforward inhibition of CA1 cells by inhibitory interneurons, or rhythmic modulation of tonic inhibition by septal afferents may give rise to the hippocampal theta rhythm. (Supported by MRC and NSERC grants).

331.15 BENZODIAZEPINE RECEPTOR ACTIVATION BY DIAZEPAM (VALIUM) DISSOCIATES AND REORGANIZES HIPPOCAMPAL EEG-BODY MOVEMENT CORRELATIONS. M.Caudarella, T.Durkin*, D.Galey*, Y.Jeantet* and R.Jaffard*, Lab. Neurobiologie: Médiateurs et Comportement, Univ.Bordeaux I, 33405 Talence Cedex France.

In Vanderwolf and co-workers' theory (Behav. Brain Sci., 1981, 4:459) the following correlations (among others) between hippocampal EEG, cholinergic mechanisms and motor behavior are postulated: 1) Walking is always accompanied by rhythmic slow-wave activity of 7-12 Hz (RSA or "theta") in the hippocampus (HPC) and is never associated with large-amplitude irregular activity (LIA) which is associated with immobility; 2) When RSA occurs during immobility or anesthesia, it has no relation to movement, is of lower frequency (4-6 Hz) and is sensitive to cholinergic blocking agents. In a series of experiments in which male BALB/cByJ mice were implanted stereotaxically with bipolar platinum electrodes in CA1 area of HPC where RSA amplitude reached 1.5 mV, the EEG spectral characteristics and the animal's motor behavior were studied while the animals walked on a 24-cm-long moving belt (speed: 2.2 cm/sec) both before and after i.p. injections of diazepam (DZ)(Valium, 2 mg/kg) or vehicle (Tween-NaCl). Most EEG analyses were carried out on-line by a Minc 11 computer. The results are surprising since DZ produced a dissociation of locomotion and RSA. Our principal results: 1) Uninjected and vehicle-inj. mice showed typical RSA (7-8 Hz) while walking; 2) Under DZ,locomotion was always accompanied by LIA, never by 7-8 Hz RSA (8 Ss); 3) Under DZ, RSA with a sharp, narrow-band peak at 4-5 Hz was associated with immobility if, and only if, the immobility immediately followed walking. This was true whether the animal itself stopped walking or the experimenter stopped the moving belt. This theta activity predominated for about 30 sec (decaying rapidly after about 10 sec) and virtually disappeared after 2 min. Thus this type of RSA may represent some kind of short-term neural trace of locomotion; 4) Scopolamine (i.p. 1 mg/kg), a cholinergic blocker, greatly reduced the DZ-induced 4-5 Hz RSA but also partially restored 7-8 Hz RSA; 5) The implication that cholinergic mechanisms underlie the DZ effects was investigated by measuring Na-dependent, high-affinity choline uptake kinetics in the HPC of 5 DZ-(2 mg/kg) and 4 vehicle-injected mice. DZ produced a highly significant but unexpected 19% decrease in choline uptake velocity. Results 4 and 5 suggest that DZ effects on HPC EEG involve changes in septo-HPC cholinergic activity; however, the precise nature of these changes requires further investigation.

331.16 THE EFFECTS OF MOVEMENT AND TENSION ON SPONTANEOUS EEG IN NORMAL HUMAN SUBJECTS. R.E. Steenhuis. Dept. of Psychology, Univ. of Western Ontario, London, Ontario, Canada, N6A 5C2.

The effects of a variety of movements, body tension and thinking about movement on spontaneous EEG were examined in six normal human subjects. The EEG recorded from the occipital (O1), vertex (Cz) and somatosensory (C3) leads with reference to linked ears (A1-A2) was acquired on-line by microcomputer. Logarithmic auto-power spectra, phase and coherence spectra between any two channels were calculated.

Activity in the alpha frequency band (AAB), peak frequency 9.96 ± 0.73 Hz (range 7.8-10.9 Hz), was recorded from all subjects at O1, Cz and C3. The maximal power was at O1 in four of the subjects and at Cz in the other two. AAB was completely suppressed by eye opening in half the subjects but only slightly suppressed in the others. In all subjects with eyes closed, movement and tension when compared to relaxed immobility resulted in a slight reduction of AAB power at O1, Cz and C3 (except at O1, in one subject). Substantial suppression of AAB by movement and tension occurred at all sites in two of the subjects who produced AAB with eyes open. Thinking about movement did not have a consistent effect on AAB. Coherence between O1 and Cz was significantly lower than between Cz and C3 ($p< 0.001$) for all conditions. The phase relationship between O1 and Cz was variable while Cz and C3 were consistently in phase.

In previous literature, mu and alpha activity have been differentiated on the basis of frequency, topography and behavioral responsiveness. The behavioral distinction between alpha and mu activity reported by other investigators was not found in the present study. Eye opening did not selectively suppress occipital AAB nor did movement selectively suppress central AAB. The fact that the largest movement related suppression of AAB occurred with eyes open suggested an interaction between visual input and motor output. Consistent with previous findings a low coherence between central and occipital activity and a high coherence between central leads supported the presence of two generators of rhythmical activity in the alpha range, one occipital and one central. (supported by NSERC grants)

331.17 TREE-STRUCTURED METHODS CLASSIFY SPATIAL PATTERNS OF BULBAR EEG AMPLITUDE. K.A.Grajski*, L.Breiman*, W. J. Freeman. (SPON:D. Weisblat). Group in Biophysics. Depts. of Statistics and Physiology. UC Berkeley. Berkeley, CA 94720.

Classification and Regression Trees (CART) are used to classify spatial patterns of EEG amplitude recorded in the olfactory bulb of rabbits. In exploring across-animal and within-trial differences, CART demonstrates the effectiveness of EEG amplitude analysis.

CART implements a classification technique to recursively partition a multivariate data space (Breiman, 1984). The partitioning process yields a series of binary trees with varying complexity. Data re-sampling methods select a tree which minimizes complexity and maximizes performance. CART makes no distributional assumptions about the data.

Oscillatory bursts of field potentials were recorded epidurally from the lateral aspect of the olfactory bulb of chronically implanted rabbits with an 8 by 8 electrode array. Each burst is displayed as a contour map of amplitude. These maps reveal active EEG focii with overall pattern unique to each animal. Bursts from each of five rabbits are represented as a 64-vector of RMS amplitudes. With data resampling, CART produces a tree with an error rate of 0-15%. By normalizing the RMS vectors, the spatial structure of a burst is enhanced; CART then selects a tree with error rate of 0-1%. These results confirm the observation that while burst amplitude may vary, its spatial structure is stable (Freeman, in preparation).

These same rabbits are classically conditioned to give a discriminative response to a CS+ odor. Bursts are recorded before and after odor presentation. The reaction time to an olfactory cue for rabbits is near one respiratory cycle. It is hypothesized that the critical information for discrimination lies in the "decisive" burst recorded just after odor presentation. Air-decisive burst pairs from CS+ trials with response and CS- trials without response are studied. Running CART with unnormalized RMS yields trees with chance levels of classification. This suggests that odor discrimination is not based on burst amplitude discrimination. With normalized RMS values, CART again produces chance level trees. This confirms that patterns of burst amplitude do not change upon odor arrival despite amplitude reduction.

In short, a powerful new classification technique confirms that spatial patterns of burst amplitude are stable and unique to each animal, but cannot characterize the informational content of a decisive burst.

331.18 COMPARISON OF THE EFFECTS OF AN ADENOSINE AGONIST AND PENTOBARBITAL ON CORTICAL BLOOD FLOW AND EEG IN RATS. L. T. Meltzer*, F. W. Marcoux, C. Christoffersen*, J. J. Cordon*, K. A. Serpa*. (Spon: J. Burchfiel) Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Adenosine and metabolically stable adenosine analogs (N^{6}- ℓ-phenylisopropyladenosine, ℓ-PIA) produce a variety of pharmacological effects. Systemic administration of ℓ-PIA induces hypotension, bradycardia, and behavioral sedation (inhibition of locomotor activity) in rodents. In the brain, adenosine and its analogs generally have inhibitory neurophysiological effects, both presynaptic and postsynaptic. In order to more completely evaluate the central effects of adenosine, we assessed the effects of ℓ-PIA on two measures of cerebral activity, local cerebral blood flow (LCBF) and electroencephalographic (EEG) activity. The effects of ℓ-PIA were compared with those of the CNS depressant, pentobarbital (PB). LCBF was measured by the hydrogen clearance method from an electrode implanted in the frontal cortex. At least three hydrogen clearance curves were taken pre-drug followed by three clearance curves at 15, 30 and 45 minutes post-drug. EEG activity was recorded differentially from screw electrodes implanted bilaterally over the frontal cortex. Electromyographic (EMG) activity was recorded differentially from bilateral temporalis muscle wire electrodes.

Both ℓ-PIA (0.1 and 0.3 mg/kg, IP and PB (30 mg/kg, IP) produced decreases in LCBF. These results are consistent with a cerebral depressant effect of both ℓ-PIA and PB, the measurement of cerebral blood flow being an indicator of cerebral metabolic rate. However, ℓ-PIA and PB produced opposite effects on EEG activity. PB (10 and 30 mg/kg, IP) produced a high-amplitude EEG, characteristic of a sedated state. In contrast, ℓ-PIA (0.1 and 0.3 mg/kg, IP) produced a low-amplitude EEG, characteristic of an awake state. However, after both drugs, the animals appeared behaviorally sedated, i.e., they lay stretched out on the cage floor, with eyes slit or closed. This represents an EEG-behavior dissociation produced by ℓ-PIA.

Thus, even though both PB and ℓ-PIA produce decreases in LCBF, consistent with cerebral depressant actions, as well as behavioral sedation, they produce opposite effects on cortical EEG. The physiological effects responsible for these differing EEG effects are not known.

331.19 COHERENCE ANALYSIS OF 100 HZ to 500 HZ ELECTRICAL ACTIVITY OF THE BRAIN. C. C. Turbes, G. T. Schneider* and R. J. Morgan. Dept. of Anatomy, Creighton Univ. Sch. of Med., Omaha, NE 68178 and Dept. of Biophysics and Physiol., Colorado State Univ., Ft. Collins, CO 80521.

In these studies special concern is given to coherence spectra of the electrical activity at 100 Hz to 500 Hz between cerebral cortical and subcortical regions of the brain. Studies involve cats with chronic implanted electrodes in areas of the cerebral cortex, septal nuclei, nucleus accumbens and amygdala. The electrodes used in these studies are stainless steel wire with 100μ to 200μ tip exposures. Electrodes with resistances of 20 MΩ or less are used. These electrodes are capable of recording both slow wave potentials and multiple unit spike potentials concurrently. These analog signals are recorded on FM tape recorder and analog to digital converted and processed with a minicomputer. Cross spectral, coherence spectral, partial coherence and cross phase spectral estimates are carried out. In these experiments, multiple unit spikes and non-unit wave potentials are found in the 100Hz to 500 Hz electrical activity. The 200Hz to 500Hz activity show a predominance of unit spike potentials. The coherence levels are enhanced between the cerebral cortex, the nucleus accumbens, and amygdala under influence of amphetamine. Increases in coherence and changes in the cross phase spectrum relates to dosage levels and repeated doses of amphetamine. Increases in coherences at 200Hz relates to distortion of cognitive behavior in the cats.

331.20 A MEASURE OF SYNCHRONY IN THE CORTICAL EEG: THE SLOW WAVE DROWSY STATE IS SLIGHTLY MORE SYNCHRONIZED HORIZONTALLY THAN THE LOW VOLTAGE FAST STATE. T.H.Bullock, G.D.Lange and M.C.McClune*. Neurobiology Unit, Scripps Inst. Oceanog. and Dept. Neurosciences, U.C.S.D., La Jolla, CA 92093.

We assess the prevailing view that the EEG is more synchronized during states of large slow wave activity than during low voltage fast activity - a view apparently based only on visual impression. EEG synchrony is taken to mean either the degree of congruence among a given population of neurons or the number of neurons showing a given degree of congruence; the latter is better for comparing regions, individuals and species. Rabbit cortical surface EEG was recorded in alert and drowsy states, partly spontaneous, partly in the hours following 10 mg/k xylazine (Rompun) i-m. Arrays of 20+ wire electrodes were implanted via small drill holes spaced 2-4 mm apart, giving many combinations at 2-17 mm separation. The common reference was in frontal sinuses. EEG was recorded in a quiet situation, 10 channels at a time, low passed to 48 Hz, digitized at 160/s, analyzed in epochs of 3.2 s by computing the FFT and the coherence for all pairs of channels, averaged over 2-4 min. As we reported before (Bullock et al.1983, Neurosc. Abstr.9:1194), coh, the coherence function, tends to fall with distance and with frequency, though neither one is a simple function. Pooling all the pairs of the same distance between cortical electrodes permitted plotting coh against distance for each frequency band. The falling curve measures the volume of tissue above a given level of coherence. For the bands 1.2-5, 5-10, 10-20, and 20-40 Hz these curves lie one under the other, in that order; they are not monotonic but appear to fall steeply within 2 mm, more slowly from 4-10 mm, possibly more rapidly above that. The distances for coh=0.5, at 1.2-5 Hz, are 8.2mm and 5.3mm in the "slow" and the "non-slow" state, in an experiment with a wide mixture of rostral, caudal, medial and lateral electrodes. The sets of points differ with better than 99.5% confidence. In this experiment there was no significant difference in the 5-10 Hz band but in the 10-20 and 20-40 Hz bands the "slow" and the "non-slow" points were different at better than 99.5% confidence level. The same distances, ca.8 and 5mm give coh=ca. 0.3 at 10-20 Hz and ca. 0.2 at 20-40 Hz. The power spectra averaged for all the epochs and electrodes in the "slow" state showed >6 dB more power at 2 Hz than in the "non-slow" state; a minor peak lies at 6 Hz in the "slow" and at 8.5 Hz in the "non-slow"; the latter had less power also in high frequency bands. We conclude that, although the popular impression is correct, the "synchronized" record is only slightly more synchronized; it is mainly different in power spectrum. A useful assay is now available to compare other states, parts of the c.n.s. and species of animals.

332.1 SYNAPTIC CONNECTIONS OF IDENTIFIED NON-PYRAMIDAL NEURONS IN MOUSE SmI CORTEX. E.L. White and G. Benshalom*, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel.

The finding that lesion-induced degeneration can be used to reliably indicate all thalamocortical (TC) synapses in layer IV of mouse somatosensory (SmI) cortex (White, 1978) underlies the present series of studies aimed at elucidating quantitative aspects of TC synaptic connections with morphologically identified neurons. Electrolytic lesions were made in the thalamus of three month-old male CD/1 mice. Four days later, brains were fixed with aldehydes, Golgi impregnated and then processed by the gold toning/deimpregnation method. Labeled neurons having somata in layer IV of the mouse posteromedial barrel subfield (PMBSF) cortex, were identified with the light microscope and then extensive portions of them were examined with the electron microscope. Results of a serial thin section analysis showed that different non-spiny multipolar cells, as identified by light microscopy, form very different patterns of synaptic connection: Some had dendrites which formed a high proportion of their synapses with TC axon terminals, whereas other cells formed only a small proportion of TC synapses with their dendrites. As for the somata of non-spiny, multipolar cells: Some formed a high proportion of TC synapses, others much less; some formed more symmetrical than asymmetrical synapses, other just the opposite. Comparisons of TC synaptic input to cell bodies and dendrites showed that some cells formed about the same proportions of TC synapses with their cell bodies as with their dendrites, whereas, for other cells, the proportion of TC synapses formed with their somata was about double that formed with their dendrites. Still other cell bodies formed far higher proportions of TC synapses than did their dendrites. That different non-spiny, multipolar cells form such heterogeneous synaptic patterns suggests that included within this classification are cells which are likely to have very different functional roles. We are currently using similar approaches to study the distribution of TC and other synapses with layer IV spiny stellate cells, in part, to determine if cells in this class are also characterized by heterogeneous synaptic patterns. Also of interest is to determine what percentage of spiny stellate cells exhibits the non-random distribution of TC synapses previously shown to characterize a single cell of this type.

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332.2 CYTOCHROME OXIDASE STAINING IN THE RAT BARREL FIELD. P.W. Land and D.J. Simons. Dept. of Anatomy and Cell Biology and Dept. of Physiology, Univ. of Pittsburgh, Sch. of Medicine, Pittsburgh, PA 15261.

Neurons in layer IV of the rodent primary somatosensory cortex are arranged in clusters, called "barrels," which are related in a one-to-one fashion to individual vibrissae on the contralateral mystacial pad. Barrels are more difficult to visualize in rats than in mice where distinct cell-sparse hollows and septa are demarcated by cell-dense sides (Welker and Woolsey, JCN, 158:437-454, 1974). In mice, high levels of the metabolic marker cytochrome oxidase are associated with barrel hollows but not sides or septa (Wong-Riley and Welt, PNAS, 77:2333-2337, 1980). The purpose of the present study was to determine whether a comparable functional subdivision exists in rats, where the barrels are cytoarchitectonically less well defined.

Normal adult rats of the Sprague-Dawley and Long-Evans strains were used. Serial sections through the brains were cut tangential to the pial surface over the barrel field and reacted with diaminobenzidine and cytochrome C (Wong-Riley, Brain Res., 171:11-28, 1979). Some of these sections also were counterstained with thionine. Cytochrome oxidase (CO) staining revealed discrete areas of high enzymatic activity which were centered upon individual barrels. Although much of the CO activity was localized to the barrel neuropil, each barrel had scattered within it a number of large, highly reactive neurons. When these cells were seen near the barrel sides they frequently exhibited proximal dendrites that were directed toward the barrel center. Rat barrels appear better defined in CO- than in Nissl-stained material in part because CO-poor zones between barrels include both the cytoarchitectonically indistinct septa and sides. Thus, patterns of neuronal activity in the rat barrel field are more precisely organized than the packing densities of the neurons.

The appearance of CO-stained barrel fields was especially striking in many Long-Evans rats where the within-row "septa" were as distinct as those between rows. In other animals, most often Sprague-Dawleys, the transition from one barrel to the next within the same row frequently was blurred.

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332.3 RECEPTIVE FIELD CHARACTERISTICS OF PRIMARY SOMATOSENSORY CORTICOPONTINE NEURONS IN THE RAT. C. E. Adams, J. K. Chapin. Univ. Tx. Hlth. Sci. Cntr., Dallas, Tx. 75235.

As part of an effort to determine what kind of information is transmitted via the cerebro-ponto-cerebellar system, we have analyzed the receptive field (RF) properties of identified corticopontine cells in the rat. Extracellular, single unit recordings in halothane anesthetized Long-Evans rats were made in layer V (1000-1700 micrometers in depth) of the forepaw and forelimb areas of the rat SI cortex and also in the dysgranular zone (DZ) lying lateral to the forelimb area. Bipolar stimulating electrodes were positioned within the basilar pons and contralateral SI cortex in order to identify, by antidromic activation, cortical neurons projecting to those areas. Anatomical studies in this laboratory have shown that callosal as well as corticopontine connections originate from widespread regions of the rat SI cortex, including forepaw/forelimb areas and the DZ. All units described were recorded in layer V along the forelimb/DZ border and were activated antidromically at a constant, short latency by basilar pontine stimulation. In addition, 17% of these same units were activated antidromically by stimulation of the homologous (forelimb/ DZ border) region of the contralateral SI cortex while 33% of the units responded orthodromically to the contralateral SI stimulation. Most of the corticopontine units in the forepaw and forelimb areas exhibited clearly definable RF's, those that did not were located within the DZ where the neurons are quite sensitive to the effects of anesthesia and do not respond to sensory stimulation. RF's of the remaining corticopontine units were primarily cutaneous in nature and heterogeneous with regard to surface topography. Recorded cells were activated by stroking the contralateral dorsal forelimb as well as by movement of multiple contralateral and ipsilateral vibrissae. One corticopontine unit responded to elbow flexion but not to cutaneous stimulation of the forelimb, indicating that this unit possessed a non-cutaneous joint RF. These observations suggest that rat SI corticopontine neurons transmit relatively complex information to the basilar pons. It is expected that future studies in the awake, freely moving rat will provide 1) a more complete assessment of SI corticopontine RF characteristics and 2) an insight into the firing patterns of this class of cortical neuron during movement. (Supported by NS-18041, AA-0390 and the Biological Humanics Foundation)

332.4 AN EFFERENT PROJECTION FROM THE PARAMEDIAN PONTINE RETICULAR FORMATION (PPRF) TO THE PREFRONTAL CORTEX IN THE BABOON. D. Riche, G. Behzadi, L.S. Calderazzo Filho and R. Guillon, Laboratoire de Physiologie Nerveuse, C.N.R.S. 91190 - Gif-sur-Yvette, France. (SPON : M. Denavit-Saubié).

On the basis of both clinical and electrophysiological observations the prefrontal cortex is involved in neural events preceding eye movements. The PPRF is also recognized to have a central role in eye movements. In the present study two young baboons were injected with horseradish peroxidase in frontal zones of the peri- and prearcuate cortex (areas 8 and 46) and a third one with a more restricted injection, located in the lower bank of the principal sulcus, in the middle third of the prearcuate area. Using the sensitive procedure of De Olmos, labeled neurons were found within the limits of the PPRF. The more rostrally labeled cells were located, ventral to the cerebral aqueduct, at the level where fibers of the trochlear nerve are easily distinguished in the ventrolateral aspect of the central gray. The extent of labeled neurons lies in the posterior region of the oculomotor complex. Some cells infiltrate between the fibers of the medial longitudinal fasciculus, mostly towards the midline. Ventrally they reached the raphe nucleus. The more caudally labeled cells were found at the level where the first neurons of the abducens nucleus can be observed. The great majority of these cells were ipsilaterally labeled, but 3 evident contralaterally labeled cells were also observed. These labeled neurons are not heavily clustered, so this connection appears to be composed of loosely grouped fibers. Contralateral labeled neurons testifie of a thin decussating fiber system across the midsagittal plane.

As this labeled area coïncides very closely with the PPRF and this connection with the pathway recently described by Leichnetz and Smith with anterograde technique (Soc. Neurosc. Abstr. 9, 749, 1983), we assume that it corresponds to that involved in horizontal eye movements and that the pontine-prefrontal pathway demonstrated here may be one of those implied in the control of conjugate eye movements.

332.5 **HYPOTHALAMIC-CORTICAL PROJECTIONS IN THE RAT.** C.B. Saper. Dept of Neurology, Washington Univ. Sch. Med., St. Louis, MO 63110

A number of studies in the last decade have demonstrated that hypothalamic neurons innervate the cerebral cortex, but this projection has not previously been studied in detail. Retrograde transport of wheat germ agglutinin-HRP conjugate was used to identify the hypothalamic neurons innervating different cortical areas. Two populations of hypothalamic-cortical projection neurons were found to innervate the entire cortical mantle ipsilaterally: 1) neurons scattered through the tuberal lateral hypothalamus (LHAt), clustering in the perifornical region, the zona incerta, and along the medial edge of the cerebral peduncle; and 2) a dense cluster of neurons in the posterior lateral hypothalamic area (LHAp), including many in the supramammillary nucleus. A crude topographic arrangement of neurons with respect to cortical field of termination was observed in the peri-fornical part of LHAt; a much more precise topographic pattern was found in LHAp. Together, these two cell groups contained 20% more labeled neurons than the magnocellular basal nucleus (MBN), and therefore constitute a major source of cortical afferents. Two much smaller hypothalamic-cortical projection cell groups were also found: 3) neurons in the fields of forel (FF) innervated only the medial and lateral frontal cortex; and 4) neurons in the tuberomammillary nucleus on each side of the brain innervated the entire cerebral cortex, bilaterally.

Anterograde autoradiographic tracer experiments showed that LHAp axons reach the cerebral cortex via two pathways: a medial path transversed the medial forebrain bundle and ran through the medial component of MBN; one branch traversed the dorsal fornix to innervate the hippocampal formation, and a second coursed over the genu of the corpus callosum to enter the cingulate bundle, from which it distributed to the cortex on the medial surface of the hemisphere. Other LHAp fibers formed a lateral pathway which ran through the lateral part of MBN into the external capsule, from which it distributed to innervate the lateral wall of the hemisphere. Fiber labeling was heaviest over layers V and VI, and to a lesser extent layer I of the cerebral cortex.

The hypothalamus provides a major source of diffuse cortical innervation, which may be underlie its involvement in generalized arousal, and a variety of specific behaviors.

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332.6 CHOLINERGIC EEG ACTIVATION OF NEOCORTEX AND HIPPOCAMPUS: ROLE OF SUBSTANTIA INNOMINATA AND MEDIAL SEPTAL-DIAGONAL BAND REGION. D. J. Stewart, D. F. MacFabe* and C. H. Vanderwolf. Dept. of Psychology, Univ. Western Ontario, London, Ontario, Canada, N6A 5C2.

Systemic injection of quinuclidinyl benzilate partially abolished low voltage fast activity (LVFA) in the neocortex of waking rats, resulting in the appearance of large irregular slow waves during Type 2 behaviors (e.g., immobility, sniffing without head movement, face washing). These slow waves did not occur during Type 1 behavior (e.g., walking, head movement). Atropine sulfate produced a similar effect but it was less potent by a factor of about 12. Injection of kainic acid into the substantia innominata: a) destroyed local cells which contain acetylcholinesterase (AChE) and reduced AChE staining in the ipsilateral neocortex; and b) produced large slow waves in the ipsilateral neocortex during Type 2 behavior but not during Type 1 behavior. These slow waves were abolished by systemic injection of pilocarpine. Kainic acid injection into the thalamus produced extensive local cell loss but failed to produce slow waves in the neocortex. The data suggest that the LVFA which is normally present in the neocortex during waking Type 2 behavior is dependent on a cholinergic input to the neocortex from the substantia innominata.

A parallel series of experiments showed that the (atropine sensitive) rhythmical slow activity (RSA) normally present during urethane anesthesia can be abolished by prior injections of ibotenic acid into the medial septal and diagonal band region. The RSA accompanying Type 1 behavior is not abolished.

Cortical slow wave recording in rats with neurotoxic lesions of the basal forebrain may provide a preparation useful in the search for pharmacological treatments of Alzheimer's disease.

This research was supported by grant A0-118 from the Natural Sciences and Engineering Research Council.

332.7 INTRINSIC CONNECTIONS OF THE SUPERIOR TEMPORAL SULCUS REGION IN THE RHESUS MONKEY. B. Seltzer and D.N. Pandya. V.A. Hospital, Bedford MA 01730.

Previous studies dealt with the architectonics and afferent cortical connections of the superior temporal sulcus (STS) (Seltzer and Pandya, '78;'84). In the present study, the intrinsic connections of different architectonic divisions of the STS were investigated using autoradiographic and HRP techniques.

In the lower bank, the middle segment (caudal areas TEa and TEm) projects to the rostral segment (rostral areas TEa and TEm) which, in turn, projects to the temporal pole (area Pro). There is also a rostral-to-caudal sequence of connections: within areas TEa and TEm, and from both of these areas to the caudal lower bank of the sulcus (area OAa, or "MT") and occipitotemporal cortex (areas OA and TEO). The lower bank of the STS also projects laterally to the inferotemporal region: from rostral sectors to area TE1; from middle sectors to areas TE2 and TE3.

In the upper bank there is a similar bidirectional flow of connections. Caudal and rostral segments of areas TPO and PGa, multimodal zones deep within the upper bank, are reciprocally interconnected. Caudal area TAa, at the outer edge of the upper bank, projects to rostral area TAa which, in turn, projects to the temporal pole (area Pro). There is also a rostral-to-caudal sequence of connections within area TAa. Finally, area TAa has additional projections: medially, to areas TPO and PGa deeper within the STS; and laterally, to subdivisions of the superior temporal gyrus: areas TS1 and Ts2 rostrally; areas Ts3 and Tpt caudally.

Rostrally-directed pathways in the STS originate principally in layer III and terminate, in columnar fashion, mainly in supragranular layers. Caudally-directed sequences, by contrast, have their cells of origin in layers V and VI and terminate in layer I of the target zone. Laterally- and medially-directed pathways have a mixed pattern: cells of origin in laminae III, V, and VI; terminations in both supra- and infragranular layers. These basic patterns resemble those previously described for both superior (Galaburda and Pandya, '83) and inferior (Rockland and Pandya, '79) temporal regions. However, in areas TPO, PGa, and IPa of the STS, cells of origin are located in both supra- and infragranular layers regardless of the direction taken by their axons.

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332.8 AUDITORY AND LIMBIC CONNECTIONS OF THE POSTERIOR INSULAR-RETROINSULAR CORTEX IN THE RABBIT. J.H. McLean, J. Johnson*, J. Keller and M.T. Shipley. Dept. of Anatomy and Cell Biology and Dept. of Neurosurgery, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0521.

Recently, the insular (IC) and retroinsular (RIC) cortex have been shown to have connections with areas of the brain involved in various sensory modalities. This cortical region also has limbic and subcortical connections. Thus IC and RIC serve as important conduits between sensory and limbic structures in the brain. In general, however, the connections IC and RIC are still poorly understood. This study has attempted to delineate the connections of posterior IC-RIC with auditory and limbic structures.

Single or multiple injections of 1% WGA-HRP totaling 50-150 nl were placed in the posterior IC-RIC (AP Level 7.0-10.0 Atlas of Urbin and Richard '72) of male albino rabbits. Animals survived 2-3 days; frozen sections were processed using the chromogen TMB to visualize HRP.

Injections in the posterior IC-RIC resulted in retrograde and anterograde label in the homologous contralateral cortex, ipsilateral insular cortex at levels rostral and caudal to the injection and in the ipsilateral association auditory cortex (AAC). Projections to and from posterior parts of the lateral nucleus of the amygdala and the entorhinal cortex as well as reciprocal connections with the dorsal, lateral and ventral portions of the medial geniculate nucleus were observed. Retrogradely labeled neurons were also present in the dorsal raphe and locus coeruleus. To determine how AAC projections terminate in the IC-RIC and if thalamic projections of AAC were similar to those of IC-RIC, WGA-HRP was also injected into the AAC region in rabbits. These injections produced anterograde labeling in deep layers of the posterior IC-RIC. Retrograde and anterograde label were observed in the region of the dorsolateral thalamus-pretectum and in nucleus suprageniculatum. These preliminary data indicate that areas of the cortex which project to the insula do not have connections in the thalamus that overlap with those from IC. Our results suggest that a sector of the insular-retroinsular cortex in the rabbit has connections which make it a potential relay for auditory input to limbic structures.

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332.9 EFFERENT CONNECTIONS OF THE MEDIAL PRECENTRAL COMPONENT OF PREFRONTAL CORTEX IN THE RAT. J.V. Corwin, R.L. Reep, A. Hashimoto and R.T. Watson. Departments of Neurology and Neuroscience, University of Florida, and Veteran's Administration Medical Center; Gainesville, Florida 32610.

Recent findings in rats indicate that unilateral cortical lesions which include the medial precentral (PCm) component of prefrontal cortex result in pronounced hemispatial neglect. A recent HRP study by us revealed strong similarities between the afferents of rodent PCm and those of arcuate cortex, a region of monkey prefrontal cortex known to be involved in attentional mechanisms. In the present study we examined the efferent connections of PCm using autoradiographic, silver degeneration, and HRP tracing techniques.

Cortical areas to which PCm projects include: contralateral PCm; ipsilateral retrosplenial, ventrolateral orbital, somatic sensory, and visual areas. Generally, layers I, III and V are the most heavily labeled.

The claustrum was labeled bilaterally but consistently heavier on the contralateral side. Fibers reach it via the corpus callosum/external capsule and terminate predominantly in its dorsal portion.

Fibers to the caudate nucleus traverse the corpus callosum and terminate in the dorsal central region. There was a moderate contralateral projection in addition to a profuse ipsilateral terminal field. A circumscribed lateral region of nucleus accumbens was usually moderately labeled.

Fibers to the thalamus gather as part of the internal capsule then traverse the thalamic reticular nucleus, in whose anterior dorsal portion some terminations appear to be made. Extensive terminal field labeling was present in these nuclei: mediodorsal (lateral portion), central lateral, ventrolateral, and gelatinosus. Moderate labeling was seen to varying degrees in several other thalamic nuclei depending on the location of the affected site within PCm.

Near its transition to the cerebral peduncle, fibers leave the internal capsule and terminate in zona incerta. A far lateral portion of deep superior colliculus usually contained a moderate amount of label, as did the ipsilateral (and to a lesser extent, contralateral) central gray of the midbrain.

The results indicate that, as for the afferents, there are strong similarities between the efferent connections of rat PCm and monkey arcuate cortex.

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332.10 EVIDENCE FOR A CORTICAL TO NUCLEUS BASALIS PROJECTION: AN ELECTRON MICROSCOPIC AXONAL TRANSPORT STUDY IN RAT. W. Lemann* and C.B. Saper (SPON: J.L.Trotter). Dept. Neurol. and Neurol. Surg., Washington Univ. Schl. Med., St. Louis, MO 63110.

The magnocellular basal nucleus (MBN) projects to all regions of cerebral cortex. Previous axonal transport studies at the light microscopic (LM) level have shown large numbers of labelled fibers in proximity to MBN neurons and their dendritic fields suggesting reciprocal innervation. We have examined this question using a new wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) method at the electron microscopic (EM) level.

WGA-HRP (10%,100nl) was injected in insular and cingulate cortex in adult rats. Following a 48 hour survival, the animals were perfused with 2.5% glutaraldehyde-0.5% paraformaldehyde. Fifty to 100 micron sections were reacted with tetramethylbenzidine (TMB) in a modification of the Mesulam method. The TMB product was stabilized by further reaction with diaminobenzidine-cobalt (DAB-Co) according to a new method described by Rye et al (J. Histochem. Cytochem., In Press). Tissue blocks were processed in the usual fashion for EM examination.

EM study showed heavy labelling of MBN perikarya and dendrites with masses of amorphous electron-dense material covering lucent rectangular spaces. These spaces are typical of the EM appearance of TMB crystals. We postulate that DAB-Co coats TMB granules which are subsequently dissolved in EM processing. Profuse labelling of myelin invested axons was seen in the vicinity of labelled MBN somata. Similar label was found in axonal terminals making synaptic contact with labelled dendrites and less frequently with labelled MBN perikarya. These results constitute evidence for a projection from cerebral cortex to MBN in rat. An alternate explanation is that the axonal terminals originate from labelled recurrent collaterals of the MBN neurons. This is unlikely because of the numerous labelled myelinated axons suggesting anterograde transport in this system.

Our data suggest that the cerebral cortex reciprocally innervates MBN cortical-projection neurons.

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332.11 QUANTITATIVE ANALYSIS OF THE MEDIODORSAL THALAMIC NUCLEUS IN THE INTACT CAT AND AFTER PREFRONTAL CORTICAL ABLATIONS. C. González* and C. Avendaño. Dept. Morfología, Fac. Medicina, Univ. Autónoma, Madrid 34, Spain.

The mediodorsal thalamic nucleus (MD) and its main cortical projection target, the prefrontal cortex (PfC), have been the subject of numerous anatomical, physiological and behavioral studies since the first description of their connectional relationship by Von Monakow (1895). Despite this interest, however, quantitative studies on these intimately related structures are still lacking.

As a first approach to this study, we used 6 adult cats to measure the volume of MD and to quantify the neuronal population in this nucleus. Also, the volume of the PfC was calculated from 6 control hemispheres. In 6 further animals the PfC was suctioned bilaterally and 3 months later the volume of the ablated cortex, the volume of the MD and the number of surviving neurons in MD were estimated. All measures were made on paraffin sections, and the values obtained were corrected for shrinkage. The intact MD had a mean volume of 24.7 mm^3 and contained on the average 228,000±13,000 (s.d.) neurons. After ablating 80% of the PfC -the neighboring cortex remaining intact- the number of neurons in MD fell to 62% of initial values, and the volume of this nucleus was reduced to about 80%. When the cortex surrounding the PfC (particularly the premotor, prelimbic and insular areas) was involved by the lesion, the changes in MD increased correspondingly, in proportion to the volume of cortex aspirated. In one case, with more than 92% of the PfC removed, plus 40 mm^3 of neighboring prelimbic and insular cortex, still more than 100,000 neurons survived in MD. These data, together with results from anterograde and retrograde tracing studies (other authors, and in preparation) show that MD is not only closely related to PfC, but also connects with other cortical regions, such as the premotor, prelimbic and insular cortices. Moreover, they provide a starting point for further quantitative studies on the development, plasticity and aging of MD and PfC, two telencephalic structures directly implicated in the most complex aspects of behavior.

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332.12 SENSORIMOTOR ASYMMETRIES AFTER UNILATERAL DM vs VPL THALAMIC LESIONS. T. Barth*, K. Holland*, and T. Schallert. Univ. of Texas at Austin, TX 78712.

Behavioral deficits that arise from damage to a given cortical area are not always evident when the associated thalamic area is damaged. The present study examined the somatosensory-motor effects of unilateral thalamic lesions in two thalamo-cortical systems of the rat: the dorsomedial (DM) thalamus - anteromedial (AM) cortex system and the ventroposterolateral (VPL) thalamus - somatosensory (SM) cortex system. We showed previously that after extensive unilateral damage to the neocortex that the capacity to localize tactile stimuli on the contralateral body surface is largely unimpaired. For example, following removal of the AM or SM cortex, animals will remove a small piece of adhesive-backed paper from the distal-radial surface of the contralateral forelimb just as rapidly as it will remove an identical stimulus from the ipsilateral forelimb, provided that the stimuli are presented one at a time. However, if the stimuli are applied simultaneously, one on each side of the midline, the ipsilateral stimulus is always removed first.

In contrast to animals with AM cortex lesions, animals with large unilateral lesions of the DM thalamus showed no ipsilateral bias in removing bilaterally-applied adhesive stimuli. Animals with unilateral lesions of VPL thalamus showed an ipsilaterally-biased asymmetry comparable to that produced by SM cortex lesions. An important feature of the sensory asymmetry following cortical or VPL thalamic lesions was its complete reversibility. Simply by incrementally increasing the size of the contralateral (C) stimulus and decreasing the size of the ipsilateral (I) stimulus (i.e., increasing the C/I ratio), the order of stimulus removal became random. In fact, when the C/I ratio was large enough the animals consistently removed the contralateral stimulus first. The minimum C/I ratio that consistently neutralized the ipsilateral bias may well be an index of the magnitude of the perceptual asymmetry caused by the brain damage. This C/I ratio in rats with VPL thalamus lesions was comparable to that in rats with SM cortex lesions, but larger than that in rats with AM cortex lesions. We are currently comparing the rate at which these C/I ratios decline with recovery.

332.13 EFFECTS OF TEMPORAL CORTICAL LESIONS ON POWER SPECTRAL ANALYSIS OF AMYGDALA ACTIVITY IN MONKEY (S. SCIUREUS). K.H. Perryman* and A. Kling. Psychiatry, VAMC Sepulveda, CA 91343 and UCLA Sch. of Med., Los Angeles, CA 90024.

This study examines the effects of bilateral lesions of inferior temporal association cortex (TE) on spectral analysis slow wave electrical activity and recording from chronically implanted electrodes in medial and lateral amygdala locations of squirrel monkeys in a) a chair and b) freely moving in a social group. In the chair condition, subjects were exposed to 1) visual stimulus consisting of a series of rear projected slides of varying subject matter, 2) a series of auditory stimuli of conspecific peeps, and 3) naturalistic stimuli.

In the freely moving condition, subjects were exposed to the same auditory stimuli and in addition recordings were correlated with naturally occurring behavioral interactions. Data were also collected in several subjects during sensory isolation. Following collection of data during the above conditions in the intact subjects, bilateral ablations of TE were carried out and the subjects re-tested for the same stimulus conditions.

Results for electrode locations in the intact subjects suggest that the lowest power was obtained under sensory isolation increasing during both visual and auditory stimulation in the chair while the highest powers for all frequency bands occurred during the freely moving condition.

Following the TE oblations there was an attenuation to visual stimulation in all electrode locations. For auditory stimulation the medial location showed a postoperative decrease in power while an increase was seen in the lateral locations. In the group situation there was a decrease noted only from the nucleus centralis while all other locations were increased. As for other behaviors in the social groups there was a general disinhibition from all locations except nucleus basal medialis and basalis anterior.

Following cortical lesions of temporal pole all electrode locations showed an attenuation in power to all stimuli in the restraint and social conditions.

These results suggest that projections from TG are excitatory to the entire amygdala while TE projections have both inhibitory and excitatory influences, the latter primarily in the lateral nuclei.

332.14 STRATEGIES FOR THREE-DIMENSIONAL IMAGING OF NEUROANATOMICAL OBJECTS RECONSTRUCTED FROM SERIAL SECTIONS. W.K. Smith, D.S. Schlusselberg, B.G. Culter* and D.J. Woodward, Dept. Cell Biology, Univ. Texas Health Science Ctr., Dallas, TX, 75235.

Ongoing studies in this laboratory have considered the question of which computer imaging strategies appear most appropriate for a range of serial reconstruction problems in the neurosciences.

In our system, data can be collected from a variety of input sources: light microscope, electron micrographs, projector, photographs, tracings and medical image data from Computed Tomography (CT) and Magnetic Resonance Imaging (MRI). Points along the perimeters of biological structures and cell positions make up the largest part of the input data. A hierarchical database and a set of routines have been designed to store and manipulate this biological information.

Stroke-vector and storage tube graphics systems were the initial devices used to display structures as sets of lines. The recently introduced raster scan graphics systems have allowed generation of much more realistic images through the display of shaded surfaces. As demostrated previously (Smith, et. al., Soc. Neurosci. 1983 Abstr. #106.5), these surfaces, which are generated using sophisticated lighting and shading algorithms, can give insight into the topology and three-dimensional relationships of anatomical objects. Transparent surfaces can also be imaged which permit the examination of interior objects or objects that would otherwise be hidden in a given scene.

Another reconstruction method is especially suited for medical image data such as that generated by CT and MRI. Since this data already contains two-dimensional picture elements (pixels), a simple extension into three dimensions allows the creation of volume elements (voxels). Fast voxel interpolation and three-dimensional display algorithms exist for this medical image data. A variation of this technique we have used includes a slab element (sloxel) for display of profiles collected from histological sections.

Our conclusion is that a number of imaging techniques must be maintained by a common database and related software packages to deal with the complexities encountered in different modes of data collection. (Support from the Biological Humanics Foundation.)

SUBCORTICAL AUDITORY PATHWAY III

333.1 **ANTEROVENTRAL COCHLEAR NUCLEUS (AVCN) PROJECTIONS TO THE INFERIOR COLLICULUS (IC) IN CAT. POSSIBLE SUBSTRATES FOR BINAURAL INTERACTIONS IN THE MIDBRAIN.** D. L. Oliver and C. Krevolin*. Department of Anatomy, The University of Connecticut Health Center, Farmington, CT 06032.

The binaural responses of neurons in the IC may involve several types of neuronal circuits. Many responses may result from projections of AVCN bushy and globular cells to the superior olivary complex whose neurons project, in turn, to the IC. However, binaural interactions also could result from direct, converging projections from the cochlear nuclei to the IC.

To investigate this hypothesis, we examined the projections of AVCN neurons and their synaptic connections in the IC with anterograde transport and LM/EM autoradiographic techniques 2-3 days after 3H-leucine injections in the AVCN. All AVCN injections produce intense labeling in the contralateral IC. Fibers terminate in the IC's central nucleus (ICC) in heavy bands or patches of labeling which parallel the fibro-dendritic laminae. Labeled afferents also extend into layers 3 and 4 of the IC's dorsal cortex (ICDC). The ventral, low frequency parts of the AVCN project to the lateral part of the ICC while more dorsomedial, higher frequency regions of the AVCN project to more medial parts of the ICC and ICDC. Projections to the ipsilateral IC are present in cases which involve the ventral and medial parts of the AVCN. Labeled afferents are largely confined to the lateral subdivision of the ipsilateral ICC and the adjacent ICDC. Preliminary electron microscopic examination of the labeled endings in the lateral ICC shows that they are quite similar on both sides. Endings contain small, round synaptic vesicles and form asymmetric contacts on small, probably distal dendrites.

Thus, the lateral subdivision of the ICC and the adjacent ICDC receive significant inputs from the AVCN on both sides. Observations of AVCN neurons labeled after HRP injections in the IC indicate that stellate cells are the likely source of both the contralateral and ipsilateral projections. These AVCN neurons may transmit information to the IC which differs markedly from that in the AVCN-to-superior olive-to-IC circuits. Neurons which respond to small interaural time differences are common in the lateral part of the IC and could be directly influenced by bilateral projections from the AVCN.

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333.2 PROJECTIONS FROM COCHLEAR NUCLEUS TO INFERIOR COLLICULUS IN NORMAL AND UNILATERAL NEONATAL COCHLEAR ABLATED GERBILS. D.R.Moore and L.M.Kitzes*. Dept. of Anatomy, University of California, Irvine, CA 92717.

The small projection from the cochlear nucleus (CN) to the ipsilateral inferior colliculus (IC) is increased in adult gerbils subjected to a neonatal ablation of the contralateral cochlea (Nordeen et al., J. Comp. Neurol., 214: 144, 1983). The distribution within the IC of the CN projection was studied in normal gerbils and in adult gerbils subjected to a unilateral ablation of the cochlea at 2 days of age. The Fink-Heimer technique for silver impregnation of degenerating axons and terminals was used. The contralateral CN of neonatally ablated animals and the left CN of adult controls were lesioned by aspiration. Survival time was 2-5 days. Adjacent frontal sections were stained for Nissl bodies and degenerating processes. Brains were studied only if there had been a discrete, large lesion of the aspirated CN and, in neonatally ablated animals, complete destruction of the cochlea and degeneration of the ipsilateral ventral CN. Silver impregnated sections were coded and the extent and density of degeneration in the IC was assessed at 1000x magnification by an observer unfamiliar with the codes.

Gradients of degeneration were seen in both IC's of all animals. In normal animals, degeneration was both more widespread and heavier in the contralateral than in the ipsilateral central nucleus of IC (ICC). Degeneration was most widespread in the rostral and lateral parts of both ICC's and in the ventral part of the contralateral ICC. Degeneration was seen in 26% of the ipsilateral and in 73% of the contralateral ICC. In ablated animals there was a much greater similarity in the extent of degeneration in the ipsilateral (57%) and contralateral (67%) ICC. The same gradients were observed as in the normal animals except that the distribution of degeneration in the ipsilateral ICC more closely resembled the normal contralateral than the normal ipsilateral profile. Although degeneration in the ICC ipsilateral to the aspirated CN was significantly ($p<0.001$) more widespread in ablated than in normal animals, the density of silver impregnated processes remained higher in the contralateral ICC.

These data demonstrate that afferent processes arising from CN are not uniformly distributed throughout the ICC. They also confirm that, in neonatally ablated gerbils, the projection to the ipsilateral IC is increased, while the projection to the contralateral IC is decreased. The altered projections are not uniformly distributed throughout the IC.

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333.3 IMMUNOCYTOCHEMICAL LOCALIZATION OF GABAERGIC ELEMENTS IN RAT INFERIOR COLLICULUS. D.E. Vetter* and E. Mugnaini. Dept. of Biobehav. Sci., Univ. of Conn., Storrs, CT 06268.

Glutamate decarboxylase (GAD) immunocytochemistry was used to assess the presence and distribution of GABAergic cells and terminals within the rat inferior colliculus (IC). Adult rats were perfused with zinc-aldehyde at pH 4.0 or 6.5 (Mugnaini & Dahl, 1983). Serial frozen sections (20 um) cut in the 3 standard planes were processed with the PAP procedure using GAD-antiserum S_3 (Oertel et al., 1981). The pattern of immunostaining identifies at least 3 regions: (A) a cortex surrounding most of IC and continuous with the sagulum; (B) a central nucleus bordered medially by the periaqueductal gray (PAG) and ventrally by the dorsal nucleus of the lateral lemniscus; and (C) a medio-dorsal region presumably corresponding to the internuclear cortex described by Cajal. Frequency of GAD-positive cell bodies and terminals was ranked from I to IV, the latter representing the highest density.

(A) The cortex contains 3 layers. The superficial layer has sparse (I) small cell bodies and medium density (II) small terminals (1.8 μm). The middle layer contains dense (IV) patches of large boutons (2.4 μm) and small-to-medium size neurons (10-20 um in average diameter) clustered with numerous GAD-negative small cell bodies. The patches measure 0.1x0.1 mm to 0.1x1.5 mm. A densely stained zone, probably continuous with the patches of the middle cortical layer, is present at the caudo-ventral border of the central nucleus. Preliminary observations suggest that the cat IC also contains patches, but that they are far less conspicuous. The deep layer is thin and has few (I) terminals and cell bodies. (B) The central nucleus displays a medium, although not homogeneous, density (II-III) of GAD-positive cells, 10-30 μm in average diameter, and terminals. (C) The superficial part of the medio-dorsal region resembles the cortex, but does not contain dense patches. The deep part, which appears continuous with PAG, contains low to medium density (I-II) of immunoreactive terminals and high density (III) of cell bodies 10-14 μm in average diameter.

Thus, GAD-immunocytochemistry indicates that GABA is an important transmitter in the rat IC. Experimental approaches combined with immunocytochemistry are necessary to distinguish between GABAergic local circuit and projection neurons and between GABAergic axon terminals of intrinsic and extrinsic origin. (Supported by NIH grant 09904.)

333.4 REGIONAL AND BILATERAL DIFFERENCES IN THE BANDING PATTERN OF LATERAL SUPERIOR OLIVARY PROJECTIONS TO THE INFERIOR COLLICULUS. A. Shneiderman and Craig K. Henkel, (SPON: P. B. Smith), Department of Anatomy, Wake Forest Univ. Med. Ctr., Winston-Salem, NC 27103

The axonal projections of the lateral superior olivary nuclei (LSO) to the inferior colliculus were examined in the cat in order to determine their distribution and configuration with respect to other afferent projections and the laminar contours of the inferior colliculus. In 20 cases tritiated leucine or WGA-HRP injections were made stereotaxically in LSO and frontal sections were processed for autoradiography or peroxidase TMB histochemistry. In each case labeled axons were organized bilaterally in bands that varied in form and position in the central nucleus of the inferior colliculus as a function of the location of the injection site. LSO fibers were distributed from an extreme dorsolateral field in the inferior colliculus in a case with an injection in the lateral tip of LSO, to the ventromedial border of the inferior colliculus in another case with an injection in the medial limb of LSO. These projections extended on both sides to the rostral process of the inferior colliculus, but not to the caudal boundary of the central nucleus. Apart from this mostly expected topography, however, the bands were oriented differently in either the ventrolateral, lateral or central and medial subdivisions of the central nucleus of the inferior colliculus [terminology by Morest and Oliver, 1984]. Furthermore, on the ipsilateral side the projection of LSO displayed alternating heavily and lightly labeled bands. The complimentary field on the opposite side sometimes appeared shifted in position and was more diffuse. The ipsilateral heavily labeled bands were usually not regularly dense along their path, but exhibited intermittent dense patches. There were also occasional examples of this on the contralateral side. It has not been established whether the patterns are in corresponding register or alternating on the two sides. While LSO projections are found in this study to overlap a broad territory of the inferior colliculus, within that territory they may be spaced in laminae or columns elaborated respectively by the bands and patches. This may reflect compartmentalization of some afferents and selective integration of others.

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333.5 SENSITIVITY OF SINGLE UNITS IN THE GERBIL INFERIOR COLLICULUS TO INTERAURAL INTENSITY DIFFERENCES AT DIFFERENT AVERAGE BINAURAL LEVELS. M.N. Semple* and L.M. Kitzes.* (SPON: E.A. Davis). Dept. Anatomy, Univ. California, Irvine, CA 92717.

The interaural intensity difference (IID) is a presumptive cue for the localization of high frequency sounds. The sensitivity of auditory neurons to IID has commonly been assessed by maintaining a fixed stimulus intensity at one ear while varying the intensity at the other (MFI) or less commonly, by increasing the intensity at one ear and decreasing the intensity by an equivalent amount at the other, thus maintaining a constant average binaural intensity (ABI). It is thought that bilaterally excited (EE) units are sensitive to changes in ABI but insensitive to changes in IID, whereas contralaterally excited/ipsilaterally inhibited (EI) units are insensitive to changes in ABI but sensitive to changes in IID. Previous studies of IID sensitivity have focused on EI units and have investigated IID sensitivity in a given neuron at only a single ABI (or MFI). In the present study of single unit responses in the central nucleus of the inferior colliculus of the gerbil, the effects of varying not only IID, but also ABI and MFI were examined. Pure tone bursts were delivered through calibrated sealed systems to animals anesthetised with Nembutal and Ketamine.

Sensitivity to IID was observed in EI, EE and PB (principally binaural) neurons. Functions of EI units fell from a maximum at IIDs with stronger contralateral stimuli (contra field) to a minimum at IIDs with stronger ipsilateral stimuli (ipsi field). The maximum discharge rate was often greater than the corresponding monaural contralateral rate, indicating facilitation at relatively low ipsilateral levels. This observation is particularly evident with MFI stimulation. The slope of the IID function was often steeper at high ABIs. EI units showed three major forms of sensitivity to increasing ABI: 1) the IID function shifted systematically toward the contra field or 2) towards the ipsi field. Shifts in either direction were often of such magnitude that, within the acoustic environment, sensitivity to IID would be limited to a restricted range of ABIs. 3) large changes in ABI produced no shift in the IID function. In PB neurons, IID functions were usually bell-shaped, with maxima at IIDs near zero, and shifted vertically as a function of ABI. Many EE units were insensitive to IID, though some revealed characteristics similar to PB or EI neurons. In such cases, there was frequently evidence of mixed excitatory and inhibitory influences arising from both ears. These results reveal: 1) that IID sensitivity occurs in neurons with diverse monaural characteristics. 2) most IID-sensitive neurons are also ABI-sensitive. 3) within a given binaural class, sensitivity to ABI is heterogeneous. (Supported by NS-17596.)

333.6 EFFECTS OF EXCITANT AMINO ACIDS ON INFERIOR COLLICULUS NEURONAL RESPONSES TO ACOUSTIC STIMULI. C.L. Faingold, W.E. Hoffmann* and D.M. Caspary. Dept. Pharmacology, Southern Illinois University Sch. of Med., Springfield, IL 62708

The neurotransmitter mechanisms which subserve auditory responsiveness in the inferior colliculus (IC) are not well understood. Earlier studies have shown that the excitant amino acids, glutamate and aspartate, will enhance the firing of many IC neurons (Curtis and Koizumi, J. Neurophysiol. 24:80, 1961; Watanabe and Simada, Jpn. J. Physiol., 23:291, 1973). This study was initiated in light of the previous iontophoretic and neurochemical studies and the recent availability of specific antagonists for the excitant amino acids. Experiments involved Sprague-Dawley rats initially anesthetized with ketamine and subsequently paralyzed with gallamine triethiodide and respired. The animal's comfort was maintained using a semichronic adapter with infiltration of local anesthetic. Characteristic frequency and response patterns of IC neurons were evaluated utilizing poststimulus time histograms. Iontophoretic application of glutamate, aspartate and the more specific agonist, N-methyl-D-aspartate (NMDA), consistently enhanced responses to acoustic stimuli with a rapid onset and offset. The threshold of IC neuronal response to acoustic stimuli could also be lowered by application of excitant amino acids. The effect of acoustically-evoked synaptically-released transmitter as well as the effect of exogenously applied excitant amino acids could be blocked by 2-amino-phosphonovalerate and other "NMDA" receptor-specific excitant amino acid antagonists. The "quisqualate" receptor-specific antagonist, glutamate diethylester, did not appear to be as effective. Baclofen, the GABA analog which is thought to decrease release of excitant amino acids, reduced the acoustically-evoked firing in IC neurons in a manner which differed in time course from that seen previously with GABA. The results to date in these experiments parallel the iontophoretic findings in cochlear nucleus with agents affecting the action of excitant amino acids which support a role of these agents in neurotransmission in cochlear nucleus (Caspary et al., Hearing Res., 4:325, 1981; Caspary et al., Hearing Res., 13:113, 1984). If the iontophoretic results in IC can be supported by additional neurochemical studies similar to those done in cochlear nucleus, this would suggest a possible role for excitant amino acids in neurotransmission in the inferior colliculus. (Supported by Deafness Research Fdn., NIH NS 15640, SIU CRC and BRSG funds.)

333.7 RESPONSES OF SPACE-SPECIFIC NEURONS IN THE OPTIC TECTUM OF THE OWL TO NARROW-BAND SOUNDS. S.E. Esterly* (SPON:J.Middlebrooks). Dept. of Neurobiology, Stanford University, Stanford,CA 94305

The auditory system analyzes binaural cues for sound localization in a frequency-specific manner. When derived from a single frequency, these cues are spatially ambiguous, since the same interaural values can arise from several locations in space. To accurately determine the location of a sound source, the auditory system must eliminate these ambiguities.

Auditory units in the optic tectum of the barn owl respond best to broadband sounds, and only when the source is located in a single, restricted spatial field. However, when tested with narrow-band (1/3 octave or tonal) stimuli under free-field conditions, those units that respond reveal the spatial ambiguities associated with frequency-specific cues. For some, discrete additional excitatory areas appear, and the locations of these accessory fields vary as a function of stimulus frequency. Although the center of the primary field, or best area, remains constant, the boundaries of this field vary as a function of stimulus frequency. When the source of a narrow-band stimulus is located in an accessory field, a small change in the center frequency may reduce the excitatory effect or inhibit the unit Moreover, when a new frequency is added to an effective narrow-band stimulus the response of the unit may be suppressed. In contrast, when the source is located in the best area, adding the same frequencies to the narrow-band stimulus facilitates the response of the unit. Thus, the accessory fields associated with frequency-specific cues are actively suppressed through cues provided by other frequencies. The best area of a unit represents the one area in space from which the cues provided by all effective frequencies are excitatory.

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333.8 **SPACE SPECIFIC NEURONS OF THE BARN OWL'S INFERIOR COLLICULUS REQUIRE ONLY TWO FREQUENCIES TO SPECIFY STIMULUS LOCATIONS.** T. Takahashi and M. Konishi. Division of Biology 216-76, Caltech, Pasadena, CA 91125.

Tonal stimuli appear to emanate from a number of plausible loci, whereas broad-band stimuli can be localized more precisely. We describe a neural correlate of this phenomenon as it is exhibited by the space specific neuron, an auditory neuron of the barn owl's inferior colliculus and present evidence that it requires only two frequencies to specify the location of a sound source. A space specific neuron is selective for interaural time differences (ITD), and interaural intensity differences (IID) which, to the owl, signify the source's azimuth and elevation respectively. The firing of a space specific neuron, therefore, represents a particular combination of IID and ITD and thus, specifies the location of a sound source.

When stimulated by its best frequency (BF) a space specific neuron discharged maximally at a number of different ITD values. The distance between the peaks equaled the stimulus period. If frequencies lower or higher than the BF were presented, the inter-peak distance, respectively, increased or decreased; however, one ITD, the "characteristic delay," always displayed a peak. Because more than one ITD causes maximal discharge, space specific neurons cannot specify a unique azimuth to a source of tonal stimuli. By contrast, when space specifc neurons were stimulated with noise (0.3-15 kHz, 3dB bandwidth), they responded maximally to only one ITD value. The response vs. ITD function displayed a major peak at the characteristic delay and smaller "side peaks," (20-60% of the major peak) at ITD values removed from the main peak by one period of the unit's BF.

A single frequency, F2, presented along with the BF suppressed side peaks as well as did noise. Not every F2, however, suppressed side peaks. An effective F2 presented along with the BF reduced the firing rate (beyond the level elicited by the BF alone) when the ITD equaled non-characteristic delays, but either had no effect or potentiated the firing (above the level elicited by the BF alone) when the ITD equaled the characteristic delay. In ITD-selective neurons whose side peaks could not be suppressed by noise, the combination of an F2 and BF elicited the same response, regardless of whether the ITD equaled a characteristic or non-characteristic delay.

333.9 TELENCEPHALIC PROJECTIONS OF THE MEDIAL GENICULATE BODY IN THE OPOSSUM (Didelphis virginiana). M. Kudo*, S. B. Frost*, K. K. Glendenning, and R. B. Masterton. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

Projections from the medial geniculate body (MG) and the related thalamic nuclei to the cortex and the basal ganglia were studied in the opossum using both the retrograde horseradish peroxidase (HRP) and ^{3}H-leucine autoradiographic methods as follows:

1) Single injections of HRP were placed in the core auditory cortex (AC) or the cortical belt surrounding AC. In each case retrogradely labeled neurons were seen mainly in the rostral two-thirds of MG. Injections in the core of AC resulted in labeled neurons in both the central and the marginal parts of MG. In contrast, injections in the cortical belt of AC produced labeled neurons chiefly in the marginal parts of MG.

2) In other cases injections of HRP into the putamen and the dorsal part of amygdala were made using micropipettes introduced through the piriform cortex to avoid possible diffusion of HRP into the neocortex. In each case a large number of labeled neurons were seen in the caudal half of MG. Labeled neurons were also seen in the nearby supragenicular and subparafascicular nuclei.

3) In still other cases, injections of ^{3}H-leucine were placed in MG. In each case, heavy terminal labeling was seen in the putamen, the amygdala and the caudal part of the caudate nucleus. Terminal label was heavy in AC following a large injection in MG; by contrast, terminal label in AC was slight following a restricted injection in the caudal part of MG.

The results suggest that there are at least two populations of neurons in MG in terms of having different projection targets: the rostral part of MG projects chiefly to auditory cortex; the caudal part of MG projects chiefly to basal ganglia. This is in good accordance with previous reports that the caudal part of MG does not degenerate following lesions in the cortex (Bodian, 1942; Diamond and Utley, 1963). However, there still remains a possibility that some neurons in MG project to both the cortex and the basal ganglia because the two populations appear to overlap at middle levels of the rostro-caudal extent of MG.

333.10 PREDICTION OF RESPONSES OF THE SQUIRREL MONKEY'S MGB CELLS TO SPECIES SPECIFIC VOCALIZATIONS BASED ON THEIR VOLTERRA KERNELS. Y. Yeshurun*, Z. Wollberg, N. Dyn* and N. Allon*. *School of Mathematical Sciences and Dept. of Zoology*, George S. Wize Faculty of Life Sciences, Tel Aviv Univ., Ramat Aviv ISRAEL.

Studies on the functions of the CNS frequently resides on the analysis of neural input output relations. However, such studies are often limited to a qualitative and subjective inspection of raw data.

System identification methods can be used to formalize the stimulus-response relations, and one of them, the Volterra approach, is employed here in order to describe the transformations taking place in neurons of the awake squirrel monkey's MGB. The inputs are natural vocalizations and reversed vocalizations, and the output is the PSTH. First and second order Volterra kernels of 41 MGB cells were calculated, where each cell is assumed to be a multi input and single output system.

In order to validate the formal representation of the system under study, the predictibility power of the model is tested. We predict responses of the cell to given stimuli (vocalizations, reversed vocalizations and pure tones), and compare these predictions to the real responses. It is found that for more then 75% of the responses, the predictions made by the model are "closer" (by our measure) to the real responses than some arbitrarily chosen responses.

We conclude that many of the MGB cells in our sample can be characterized by their Volterra kernels, and further research on the cells' functional role can be based on these kernels.

333.11 LOCALIZATION OF VIRTUAL SOUND IMAGES BY BARN OWLS. A. Moiseff and M. Konishi, California Institute of Technology, Pasadena, CA 91125.

The owl's auditory system analyzes interaural time and intensity differences in separate, parallel pathways for localization of sound. This report presents behavioral evidence for the separate use of the interaural cues for determining sound azimuth and elevation.

Two tame barn owls (Tyto alba) were outfitted with earphones for dichotic presentation of sound, a 100-msec. duration noise burst. Computer controlled phase shifter and attenuators varied, respectively, interaural time and intensity differences. The owl's head-orienting response to sound allowed the measurement of perceived locations by the search coil technique. The results confirm our earlier finding that perceived azimuth locations are strongly correlated to interaural time differences (slope of the linear equation describing the data is: 0.37 degrees of azimuth/μsec with a correlation coefficient of 0.94). There is a small elevational component to the response to interaural time difference (-0.06 degrees of elevation/μsec with a correlation coefficient of -0.21).

The owls responded to stimuli containing only interaural intensity differences by orienting their heads in elevation. The linear relationship between elevational location and interaural intensity difference is 1.36 degree of elevation/dB (correlation coefficient of 0.86). There is a slight dependence of azimuth on the interaural intensity difference (0.58 degrees of azimuth/dB with a correlation coefficient of 0.26). The average intensity of the binaural stimulus had no effect on sound localization.

From the above observations we hypothesize that the anatomical and physiological separation of time and intensity sensitive pathways of the owl's auditory brainstem manifest themselves as the differential behavioral use of these binaural cues.

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333.12 INTENSITY DEPENDENT LATENCY: A POSSIBLE TEMPORAL MECHANISM FOR SOUND LOCALIZATION IN THE MUSTACHE BAT. C.L. Resler* and G.D. Pollak. Dept. of Zoology, Univ. of Texas, Austin TX 78712.

A free field sound stimulus provides an interaural intensity difference of between 0-30 dB at the tympani, depending on the location of the source. Single unit studies in the inferior colliculus (IC) of bats show a latency decrease of up to 5 msec with a 10 dB increase in sound intensity. Therefore a latency difference between ascending inputs may encode sound direction. This intensity derived time cue greatly exceeds the physical time cue provided by the distance between the two ears, and was shown to encode direction as effectively as spike count in a locust (Morchen et al., Naturwissenschaften 65:656-657, 1978).

To determine the effect of time cues of this magnitude on EE and EI units in the IC of the mustache bat (Pteronotus parnellii), dichotic stimuli were presented with speakers sealed to each pinna with ear mold compound. Sinusoidally frequency modulated (SFM) tone bursts were presented which evoked phase-locked responses to the modulating waveform. The intensities at the two ears were kept constant while the relative time (phase) of the modulating waveform was varied, simulating relative latency differences. Time shifts as small as 2-3 msec readily affected response rates in both EE and EI units.

The result suggests that temporal events generated by interaural intensity differences influence binaural units and may play an important role in sound localization.

333.13 LOW FREQUENCY AUDITORY SENSITIVITY OF THE PALLID BAT, ANTROZOUS PALLIDUS. P.E. Brown*[1], P.M. Narins[1] and A.D. Grinnell[2]. Dept. of Biology[1] and Jerry Lewis Neuromuscular Research Center[2], UCLA, Los Angeles, CA 90024.

Echolocating bats are known for their high-frequency auditory capabilities, matching the ultrasonic frequencies in their orientation sounds. Some, however, exhibit behavior that suggests sensitivity at low frequencies as well, e.g., in capture of insects or calling frogs. Using behavioral techniques, Poussin and Simmons (JASA 72, 340, 1982) have documented a peak of low frequency sensitivity at about 1 kHz in Eptesicus fuscus. Pallid bats, Antrozous pallidus, which use high frequency signals for orientation, appear to utilize low frequency prey-produced sounds while foraging for insects on or near the ground. We now present neurophysiological evidence that bats of this species detect sounds as low as 1 kHz, and are extremely sensitive at 9-11 kHz.

Adult pallid bats were anesthesized (Nembutal) and the inferior colliculus (IC) exposed. Tungsten electrodes were used to make multi-unit recordings from known depths below the surface of the IC. Tone bursts for which the frequency was incremented in 100 Hz steps were presented to the bat via free-field calibrated loudspeakers (an ADS-300 with output equalized +5 dB from 200 Hz to 20 kHz and a Polaroid ultrasonic transducer from 15 kHz to 90 kHz). For each frequency tested, the threshold intensity for multi-unit responses was determined. The frequency of peak sensitivity increased systematically with depth of electrode penetration. At or near the surface, peak sensitivity was approximately 0-10 dB SPL at 9-11 kHz. At greater depths, comparable sensitivity was seen at frequencies as high as 40-50 kHz. Near the surface, threshold rose to approximately 70 dB SPL at 2 kHz. Although no separate low frequency peak of sensitivity was seen, our results are consistent with the bats' passive use of lower frequencies in insect capture.

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333.14 SOUND LOCALIZATION ALONG THE VERTICAL MIDLINE IN THE BAT:THE ROLE OF BINAURAL SPECTRAL CUES. Z.M. Fuzessery and G.D. Pollak. Dept. Zoology, Univ. Texas, Austin, TX 78712. The directional selectivity of the external ears of the mustache bat exhibit a pronounced frequency dependence when measured at frequencies in the bat's echolocation pulse. The most pronounced shift occurs near 60 and 90 kHz, where directionality changes about 40 degrees in elevation. Using a combined closed and free-field stimulation paradigm to examine both the binaural response properties and spatial selectivity of single neurons, it was found that neurons tuned to these frequencies exhibit a similar shift, demonstating that their elevational sensitivity is conferred by their frequency selectivity. Their azimuthal sensitivity is dictated by their sensitivity to interaural intensity disparities (IIDs). Their spectral and binaural response properties, combined, systematically shift spatial selectivity along the horizontal and vertical axes.

A shift in azimuthal sensitivity is most pronounced in neurons which receive binaural input, and whose responses are facilitated at certain IIDs. Their degree of spatial selectivity was influenced by several factors, among them the magnitude of facilitation, the IID range over which they were facilitated, and the differences in their monaural excitatory thresholds. Some neurons exhibited a facilitated response at some IID values, and inhibition at other values. These properties further enhanced spatial selectivity, and appeared to act as a form of spatial contrast enhancement. Equally selective were neurons unresponsive to monaural stimulation, and which were excited only by binaural stimuli presented over a narrow range of IID values. Neurons that were facilitated maximally at an IID of zero were most sensitive to sounds on the vertical midline; in some cases, they were unresponsive to sounds more than 15 degrees to either side of the midline. These neurons shifted their elevational selectivity systematically as a function of their frequency tuning. Neurons tuned near 60 kHz were selective for sounds at the intersection of the horizontal and vertical midlines, those tuned near 90 kHz, for sounds 40 degrees lower in elevation. These studies provide physiological evidence that the bat may use binaural spectral cues to localize sound along the vertical midline.

333.15 ENCODING OF AZIMUTH BY ISOFREQUENCY E-I UNITS IN THE MUSTACHE BAT INFERIOR COLLICULUS. J.J. Wenstrup, Z.M. Fuzessery, and G.D. Pollak. Dept. of Zoology, Univ. of Texas, Austin, TX, 78712.

The directionality of the external ear in mammals can exert a strong, frequency-dependent influence upon the spatial selectivity of central auditory neurons. Given this influence of the external ear, what is the contribution of binaural interactions in further shaping the spatial selectivity of auditory neurons?

In the inferior colliculus of the mustache bat (Pteronotus parnellii), we made a detailed study of single units which are sensitive to interaural intensity disparities (E-I units), and which are tuned to the same frequency (corresponding to the 60 kHz component of the bat's echolocation signal). The binaural response properties of these units, examined using dichotic stimuli, were compared to their responses to sounds at fixed positions along the azimuth, using free field stimuli. Each unit was tested over a wide range of interaural intensity disparities and absolute intensities.

We find that all 60 kHz E-I units are most sensitive, and respond best, to sounds originating in the contralateral field, about 30 degrees off the vertical midline. However, the ipsilateral border of a unit's receptive field was primarily determined by the particular interaural intensity disparity at which inhibition occurs (termed the inhibitory threshold). The strength of the inhibition influences how sharply the ipsilateral border was defined. At higher stimulus intensities, the spatial selectivity of E-I neurons further depends upon how inhibitory thresholds change with increasing intensity, and upon the monaural intensity-rate function for contralateral sound.

These studies indicate that a group of E-I cells, tuned to the same frequency, may encode azimuthal position by differences in the borders of their receptive fields, based upon differences in inhibitory thresholds. The activity within a population of E-I cells will therefore change according to the azimuthal position of a sound. We have previously shown that inhibitory thresholds are organized in the 60 kHz region of the mustache bat inferior colliculus. Our results here suggest that this organization may be a systematic representation of azimuthal position.

333.16 CONVERGENCE OF AUDITORY AND LATERAL LINE PROCESSING IN THE TORUS SEMICIRCULARIS OF THE TROUT (Salmo gairdneri). Nico A.M. Schellart*, Ruud C.V.J. Zweijpfenning* and Loek J.A. Nederstigt* (SPON: European Neuroscience Association), Lab. of Medical Physics, Herengracht 196, 1016 BS Amsterdam, The Netherlands.

Electrophysiological (Schellart, Neurosci. Lett., 42, 1983, 39-44) and neuroanatomical (De Wolf, Schellart and Hoogland, Neurosci. Lett., 38, 1983, 209-213) studies revealed that the torus semicircularis in the midbrain of the trout processes acousticolateral information. This paper describes the characteristics of single unit responses to auditory and lateral line stimulation. The stimuli were tone bursts (720 msec on and 720 msec off), whose frequency was swept from 50 to 650 Hz within 180 sec. At 2.5 Pa sound pressure of the stimulus, measured in the stomach, the frequency dependent displacement amplitude of the labyrinth was around 400 nm. The threshold of most units was near 0.1 Pa.

The high frequency or auditory (A) units (33%, N=66) are exclusively sensitive to frequencies higher than 125 Hz. The low frequency or lateral line (L, 12%) units respond to tones up to 125 Hz. The third type, the broadband (B, 55%) unit, shares its properties with the former two types. It is innervated by either the auditory system alone or by both systems. Taking the three types together, the ON units are most common (65%), the OFF units rare (10%) and mostly found among A units. The remaining ON/OFF units are all B units. They show often a multimodal spike density versus frequency characteristic. The generally higher complexity of the behaviour of the B units suggests simultaneous input from distinct types of otolith hair cells often combined with lateral line input. Most units give tonic on responses with a phasic onset of the response. For low frequencies the latency is on the average two times longer than for high frequencies. Some units show habituation and only sensitive ones exhibit phase-locking. The A units are uniformly distributed over the torus. The L and B units are localized in the caudal part of the torus, which has been confirmed by a preliminary ^{3}H-deoxyglucose study. Autoradiographic selectivity was realized by limiting the frequency range of the stimulus combined with destruction of either the labyrinths or the lateral lines.

VESTIBULAR SYSTEM II

334.1 SEMICIRCULAR CANAL AFFERENT PROJECTION IN THE VESTIBULAR NUCLEI OF THE BULLFROG. V. Honrubia, C. Suarez*, A. Kuruvilla*, I. R. Schwartz and S. Sitko*. Div. of Head and Neck Surgery, UCLA School of Med., Los Angeles, CA 90024.

The projection in the CNS of fibers from each of the three vestibular cristae was studied after horseradish-peroxidase (HRP) labeling of their individual nerve bundles. Fifteen single neurons physiologically identified from the anterior canal were also labeled.

In the vestibular root the primary fibers from the anterior crista form a shell in the more rostral side with the larger fibers located dorsally and the smaller ventrally. Horizontal and posterior cristae fibers are arranged in the same manner but more centrally in the nerve. The central vestibular tract is located lateral to the vestibular nuclei throughout their length. In this track the ascending and descending secondary fibers from the anterior crista course in a position ventral to the fibers from the horizontal and posterior cristae. The position of the thin fibers in the tract is lateral to that of the thick fibers for each organ. At irregular intervals each fiber gives medially directed tertiary fibers. The five vestibular nuclei (cerebellar, superior, medial, ventral and descending) receive innervation from the three cristae. Thin fibers' tertiary branches run a tortuous course within the nuclei and have numerous boutons en passant. The tertiary branches of thick neurons run a more direct course but have no boutons.

The medial vestibular nucleus receives only small nerve endings, mainly from the thin crista fibers. The descending nucleus receives thick and thin fibers from all the cristae. The crista innervation in both nuclei is greatest in the rostral and caudal poles. The lateral vestibular nucleus is the most profusely innervated by both thick and thin fibers. Here fibers from the anterior canal form a lateral-medial-directed strip in the ventral side. Fibers from the horizontal and posterior cristae form parallel but overlapping strips in more dorsal positions.

There are several differences in the projection of thick and thin fibers from the anterior crista. The number of terminal branches from the thick fibers is more numerous. One thick fiber can provide more than 200 terminal branches, half of them in the ventral nucleus. Thick fibers do not project in the medial nuclei and are more numerous in the reticular formation and cerebellum.

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334.2 SEMICIRCULAR CANAL AFFERENTS IN THE BULLFROG: PHYSIOLOGICAL CHARACTERISTICS VS. CELLULAR MORPHOLOGY OF HRP-IDENTIFIED NEURONS. S. Sitko*, V. Honrubia, A. Pereda*, C. Suarez* and I. R. Schwartz (SPON: D.Strelioff). Div. of Head and Neck Surgery, UCLA School of Med., Los Angeles, CA 90024.

Intra-axonal recording and subsequent labeling with horseradish peroxidase (HRP) was performed on anterior semicircular canal afferent fibers of the bullfrog vestibular nerve. Morphometric data were obtained from the fibers classified on the basis of the coefficient of variation (CV) of the resting discharge activity.

Previously, we determined that the sensitivity of primary afferents for physiological stimuli ranges from 7 to less than 1 spike/sec/deg/sec/sec. These values are correlated with the CV (and hence regularity) of the resting discharge activity. Fibers with higher CV (>0.5) have greater sensitivity and the largest axon diameters (7-17μ) and somata volumes (0.1-0.6 mm^{2}). It was also shown that the peripheral projections of the irregular neurons extend to the central regions of the sensory epithelium in the crista.

The anterior semicircular nerve branch in the bullfrog contains approximately 1100 fibers. Axonal diameters range from 1-17μ in size, forming a continuous distribution, with the number of fibers increasing exponentially with decreasing axon diameter. We have determined that the aforementioned large, "irregular" fibers represent less than 10% of the total population.

Recently, we have succeeded in labeling single anterior semicircular canal afferents characterized by a more regular spontaneous discharge activity pattern. These neurons were selected for having CV values less than 0.5, with the lowest value being 0.2. These cells are physically smaller, having 2-5μ-diameter axons and somal volumes ranging from 0.025-0.110 mm^{2}. The neurons of this sample with a lower CV represent about 30% of the total population.

We have not yet succeeded in staining single fibers whose CV's are less than 0.2, although complete physiological data have been obtained from fibers with CV values extending down to 0.09. By elimination, we propose that these fibers represent the smallest semicircular canal neurons, with axons less than 2μ in diameter and somata of less than 0.035 mm^{2} in volume. These fibers are the most numerous, comprising 60% of the fiber population.

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334.3 ARBORIZATION PATTERNS AND ULTRASTRUCTURAL CHARACTERISTICS OF SYNAPTIC MORPHOLOGY IN THE FROG CRISTA. I.R. Schwartz, V. Honrubia, S. Sitko* and C. Suarez*. Head & Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024.

Two different patterns of innervation are associated with large (>7μ) (LF) and medium (>3-≤7μ) (MF) diameter fibers to the frog cristae. LFs, filled with horseradish peroxidase (HRP) by both extracellular and intracellular injection, project to the center of the crista along its longitudinal extent; average 21.5/bundle in each of the four bundles of the vestibular nerve anterior branch which approach the crista; make up 17.5% of the two central bundles innervating the middle of the crista, and 4.4% of the two lateral bundles which carry the majority of the smallest fibers (SF) (≤3μ) that distribute to the crista ends. LFs in all bundles turn vertically upward at the base of the crista to approach the bases of the hair cells (HC), then divide into several large branches which in turn give off several smaller branches. Branches of LFs from the lateral bundles turn towards the middle of the crista. LFs contact all HCs in their projection region, and each HC may receive synapses from more than one branch. HCs in the center of the region receive the most branches. Both large and small diameter branches combine to form a lacy cup around the HC base and send fingers up its sides. Ultrastructurally the largest diameter fibers and terminals contain both a large core of flocculent material surrounded by scattered mitochondria and clusters of synaptic vesicles beneath the membrane. LFs are frequently found apposed to HC "ribbon" synapses, i.e. those with presynaptic dense bodies. In some cases the outer leaflets of membranes of the HC and LF, or its synaptic terminal, are closely apposed to form "tight" junctions.

MFs approach the cristae in all nerve bundles. At the base of the HCs, HRP filled MFs are seen to turn and run a slightly tortuous, but unbranched, centrally directed course along the length of the crista, contacting in excess of 20 HCs. As yet there is no ultrastructural basis for distinguishing between the boutons arising from MFs and LFs. Small boutons are found contacting both large fibers and HCs. Some of the smaller boutons are observed as extensions of thin branches of the largest fibers. The termination pattern of extracellularly injected SFs have been unobservable light microscopically. There are no obvious ultrastructural differences between the small synaptic terminals found at the crista ends, where the SFs predominate, and those found at the center of the crista; but LFs and large terminals are rare at the ends.

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334.4 CHANGES IN THE CHARACTERISTICS OF THE CAT'S VOR INDUCED BY UNILATERAL AND BILATERAL LABYRINTHECTOMY. K. Garland*, J. Marco* and V. Honrubia. (SPON: C.H. Sawyer). Div. of Head and Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024.

The dynamic changes of the vestibulo-ocular reflex (VOR) response after surgically produced unilateral labyrinthectomy and drug-induced ototoxicity were studied in cats. The test series included both sinusoidal (0.0125-1.6 Hz) and impulsive (30 and 50 deg/sec, CW and CCW) stimulation of the horizontal canals. The cats were tested prior to, directly following, and at various intervals after treatment.

The results of sinusoidal stimulation of 5 unilaterally labyrinthectomized cats in the immediate postoperative period (1-5 days) showed both decreased gain (peak slow phase eye velocity/peak chair velocity) and a marked asymmetry reflected in a stronger response during ampullopetal than ampullofugal stimulation of the functional canal. Results from impulsive tests showed a decrease in gain and time constant values for both CW and CCW directions; the effects were less significant during ampullopetal stimulation. There was a progressive and significant recovery of response in the 4-week postoperative period.

Bilateral labyrinthectomy was produced in 6 cats by means of the ototoxic drug gentamicin (40 mg/kg IM daily for 14 days). Although individual cats varied in their susceptibility to the drug, there was minimal or no effect for the first 7-10 days. After day 14, ototoxic effects, including ataxia and reduced VOR gain, were evident. As the ototoxicity progressed, gain continued to decrease, the change being more significant initially at the lower frequencies.

The temporal course of the response changes observed in these studies can be described using the parameters of a simplified pendulum model of vestibular function. There was a decrease in both the sensitivity coefficient and the time constant of the model. These data are similar to those obtained in our laboratory from comparable groups of human subjects.

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334.5 CANAL AFFERENT INNERVATION PATTERNS INVESTIGATED WITH LUCIFER-YELLOW DYE TRACING. S.F. Myers, E.R. Lewis, J. Caston*. Electronics Research Lab., Univ. of California, Berkeley, CA 94720.

Lucifer yellow has advantages over other intracellular tracer molecules in the ease of iontophoresis which allows even the smallest fibers to be filled (<4μm). Its bright fluorescence at the light microscopic level also allows the labeled nerve fiber to be quickly traced and the innervation pattern to be determined.

Rana catesbeinana and Rana temporia served as the experimental animals. The frogs were anesthetized with Sodium Pentobarbitol (0.12ml/100g). A small incision was then made in the mucosa of the roof of the mouth and a hole drilled through the cartilage and bone to expose the intralabyrinthine course of the nerves to the anterior and laterial cristae ampullari. Dye-filling axons from this approach was found to result in a brighter fluorescence of the terminal arborizations than if the eighth nerve were penetrated intra-cranially at the junction with the brainstem. After dye-filling a unit, the tissues were fixed in 10% formalin, dehydrated in alcohol, cleared in methyl salicylate and viewed on a Ziess fluorescence microscope.

Labeled nerve fibers ranged in diameter from 2.4 to 8.0 μm. Fibers less than 4 μm in diameter were found to innervate all regions of the nuroepithelium and to contact 8 - 15 hair cells. Larger diameter fibers of 6 - 8 μm have only been found to innervate the central portion (middle 50%) of the anterior crista ampullaris or the analogous portion of the lateral ampulla's half-crista. This finding is in agreement with those of Gacek and Rasmussen (Anat. Rec. 139, 455-463, 1961) and Dunn (J. Comp. Neurol. 182, 621-636, 1978) who found the larger diameter fibers to predominate in the central portion of the crista. The fibers innervating the central region of the cristae also had fairly extensive innervation patterns extending along the crista from 20 to 50% of its total length. The most extensive innervation patterns were found in the lateral ampulla's half-crista which may correlate with Peterka's findings that afferents form the lateral ampulla were generally more sensitive than those of the anterior ampulla (Soc. Neurosci. Abs. 6, 223, 1980).

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334.6 BIOCHEMICAL STUDIES ON THE CHOLINERGIC NEUROTRANSMISSION OF THE FROG VESTIBULE. G. Meza, P. Cuadros* and I. Lopez *
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Efferent neurotransmission in the frog vestibule is possibly cholinergic as evidenced by electrophysiological and histochemical techniques. However, no biochemical approach has been explored to assess this assumption. Some of the biochemical criteria often used in trying to implicate a substance as a neurotransmitter candidate are the demonstration of its synthesis and of the presence of its inactivating mechanism. Acetylcholine (Ach) is synthesized with the participation of choline acetyltransfer ase (ChAT) and its removal from the vicinity of the synapsis carried out through its hydrolisis by acetylcholinesterase (AchE). Choline, product of this hydrolisis, is rapidly taken up by the presynapsis and used for Ach synthesis. Therefore as a mean to demonstrate Ach participation in neurotransmission we decided to measure ChAT, AchE and choline transport in the frog isolated vestibule.
The complete vestibule was dissected out from adult frogs (Rana pipiens). ChAT was measured by the Fonnum method using ^{3}H-AcetylCoA as substrate, in homogenates of the frog tissue AchE was determined in a membrane fraction of the frog vestibule by the Ellman method following the hydrolisis of acetylthiocholine used as substrate at 412 mn in the spectrophotometer. Choline transport was assessed by measuring the uptake of ^{3}H-choline (0.5 uM) of whole frog vestibule incubated in frog Ringer solution either in the presence of Na or when this ion was substituted by choline chloride.
All incubations took place at 30°C.
ChAT activity was of 0.06 nmoles/min/mg protein and it was depressed by 90% when measured in the absence of chloride.
AchE was of 0.01 nmoles/min/mg protein and it was inhibited by 100% when incubated with 10 uM eserine.
^{3}H-choline was accumulated by frog vestibule 10-fold in an hour and it was highly depressed when Na was omitted from the incubation medium or when it took place at 4°C.
These evidences support the involvement of acetylcholine in neurotransmission in the frog vestibule. Experiments aimed to locate the cells responsible for the activities observed performed in distinct organs composing the frog vestibule are presently underway.

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334.7 MORPHOLOGY OF VESTIBULAR COMMISSURAL PATHWAYS AND NEURONS WHICH RECEIVE VESTIBULAR COMMISSURAL INPUTS. R. Balice-Gordon and R. A. McCrea, Committee on Neurobiology, Univ. of Chicago, Chicago, IL 60637.

The origin, trajectory and terminations of vestibular commissural neurons were studied in the bullfrog, Rana catesbeiana, with localized injections of WGA-HRP into the vestibular nucleus (VNuc) and by intracellular injections of HRP into vestibular neurons. The results of retrograde tracer experiments suggested that vestibular commissural pathways arise from small to medium sized cells located in the medial VNuc, the ventromedial portion of the lateral VNuc and to a lesser extent the inferior and superior VNuc. Commissural axons crossed the midline dorsally and entered the contralateral VNuc medially. A second group of commissural axons crossed the midline more ventrally, coursed dorsolaterally toward the contralateral VNuc, and entered them ventromedially. Commissural axons terminated in all portions of the contralateral VNuc. The heaviest terminations were in the medial VNuc and lateral VNuc, particularly in those areas where commissural neurons were located.

We have studied the morphology of vestibular neurons which receive commissural inputs by injecting HRP into them after their intracellular responses were recorded following electrical stimulation of the ipsilateral and contralateral vestibular nerves. So far we have injected neurons in the medial and lateral VNuc which receive commissural inputs. All of the vestibular neurons which received commissural inputs also received monosynaptic inputs from the ipsilateral nerve. Most of the commissural inputs we have observed were EPSPs evoked at latencies from 4 to 15 msec.

At least two morphological types of cells receive commissural inputs. The first type were large neurons in the lateral VNuc whose axons projected ipsilaterally and caudal in the lateral vestibulospinal tract. In most cases, the axons of these cells were not observed to collateralize within the brainstem, although in one instance collateral terminations were observed in the facial motor nucleus. The second type of neurons had axons which crossed the midline, bifurcated, and projected in the MLF. Finally, we have injected neurons with medium sized somata in medial VNuc and ventromedial lateral VNuc whose axons appear to cross the midline. In some cases, the axons were observed to collateralize within the ipsilateral VNuc. Although we have not been able to follow the axons of these cells to their terminations in the contralateral VNuc, their location, size and trajectories suggest that they generate commissural pathways.

334.8 PROJECTIONS OF INDIVIDUAL OTOLITH PRIMARY AFFERENTS IN THE GERBIL. A.A. Perachio, G.A. Kevetter, and M.J. Correia. Depts. Otolaryng., Physiol & Biophysics, Anat., Marine Biomedical Institute, Univ. Texas Medical Branch, Galveston, TX 77550-2778.

The central distribution of vestibular primary afferents is divided into two major branches that have been illustrated both in earlier studies of the entire system in Golgi material (Lorente de Nó, Laryngoscope, 43, 1933:1-38) and more recently in investigations of the projections of individual HRP-filled semicircular canal afferents (Mannen et al, Proc. Japan Acad. 58, Ser. B, 1982:237-242). The morphological characteristics of primary vestibular afferents associated with the otolith organs were examined in gerbils with intra-axonal injection of HRP. Primary otolith afferent neurons were physiologically identified by their responses to changes in static head tilt position and an insensitivity to purely angular head acceleration. Neuronal activity was generally classified in terms of the regularity of the discharge rate measured while the animal's head was held so as to position the lateral semicircular canals co-planar with the earth-horizontal. The average areal dimensions of identified otolith neuronal cell bodies were $\bar{X}\pm SD$ 368.7±127.5 μm^2, n=10. Irregularly firing neurons were generally larger ($\bar{X}\pm SD$ 412.1±118.5 μm^2, n=7), than regularly discharging neurons ($\bar{X}\pm SD$ 267.0±94.7 μm^2, n=3). Central projections of some of these neurons could be traced in both ascending and descending branches. Extensive collateralization was noted. In the descending branch, collaterals typically derived at right angles from a larger diameter fiber that coarsed within a fascicle of vestibular afferents along the longitudinal brain stem axis. These collaterals extended toward the medial vestibular nucleus while the larger fiber continued caudally within the descending nucleus. Fibers from individual afferents were traced to arborizations that resembled terminal fields in the following vestibular areas: lateral, medial and descending nuclei. Within a given nucleus, separate areas may receive projections from collaterals of the ascending and descending branches. Therefore, the otolith afferents, like the semicircular canal neurons can individually project to multiple sites in different vestibular nuclei.

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334.9 PRIMARY AFFERENT AND VESTIBULAR EFFERENT INPUTS TO THE INTERSTITIAL NUCLEUS OF THE VESTIBULAR NERVE. D.W. Jensen, J. Goldberg, and M. Igarashi. Program in Neuroscience and Dept. Otorhinolaryngology, Baylor College of Medicine, Houston, TX. 77030.

The interstitial nucleus of the vestibular nerve, located in the vestibular nerve root in the brainstem, is a distinct cell group that appears in all vertebrates, from cyclostomes to man (Mehler, Prog. Br. Res. 37: 55, 1972; Brodal and Sadjapour, J. Hirnforschung, 10: 299, 1968). We are using guinea pigs to study the anatomy and physiology of this nucleus, about which very little is known.

We have confirmed that the interstitial nucleus of the vestibular nerve (INV) receives primary afferent vestibular input, by neuroanatomical and, for the first time, electrophysiological techniques. Injections of horseradish peroxidase or tritiated leucine into the vestibular ganglion resulted in labeling in the INV. Terminal degeneration by Fink-Heimer and cupric silver methods was also observed in the INV after vestibular nerve ganglionectomy. In all cases, the vestibular nerve input to the INV appeared to be as heavy as the most dense input to the vestibular nuclei.

Functional vestibular nerve input to INV neurons is supported by electrophysiological data. Bipolar 0.05 msec. current pulses applied to the superior branch of the vestibular nerve evoked field potentials and monosynaptic spike responses in the INV neurons at a 0.95 msec. latency compared to 1.05 msec. in the MVN neurons (located more medially). Electrical thresholds for the INV field potentials and spikes were the same as for the N_1 field potential in the vestibular nuclei.

In addition, acetylcholinesterase (AChE) histochemistry has revealed a rich input to the INV from the efferent bundle of the VIIIth nerve as it travels out to the inner ear. The INV receives collaterals from an AChE positive bundle, which is the ventralmost of the vestibular nerve rootlets in the brainstem, and just ventral to the INV.

We hypothesize that the INV receives, in addition to primary afferent input, an input from vestibular centrifugal neurons.

334.10 VERTICAL EYE MOVEMENTS AND VERTICAL SEMICIRCULAR CANAL RESPONSES IN CAT DURING NORMAL PITCH AND DURING PITCH WITH THE ANIMAL POSITIONED ON ITS SIDE. D.L. Tomko, C. Wall and F.R. Robinson. Depts. of Physiol. & Otolaryngol., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

During naturally-occurring, oscillatory pitch movements of the head (earth-parallel pitch), vertical semicircular canal stimuli are paired with otolithic stimuli because head position changes with respect to the gravity ('g') vector. Typically, however, in studies both of vertical eye movements, and of eighth-nerve afferents innervating vertical canals, the pitch stimulus is delivered with the animal lying on its side (that is, activating the vertical canals with a rotational acceleration, but not activating the otoliths as they normally would be by earth-parallel pitch). It is already known that the vertical eye movements elicited by pitch differ from the horizontal eye movements elicited by yaw; the vertical vestibulo-ocular reflex (VVOR) elicited by pitch rotations while an animal is lying on its side (earth-perpendicular pitch) and vertical optokinetic nystagmus (VOKN) are not symmetric as are horizontal ones (Anderson, Dev. Neurosci., 1981, 12:395-401; Matsuo et al., Brain Res., 1979, 176:159-164). Furthermore, the known linear-acceleration sensitivity of canal primary afferents (Perachio & Correia, 1983, 280: 287-298) might affect the dynamic response properties of vertical canal afferents during earth-parallel pitch.

We have tested first, whether in the cat the VVOR elicited by earth-parallel pitch (i.e. changing the position of the head re the 'g' vector) would differ from that elicited by earth-vertical pitch (i.e. with no change in the position of the head re the 'g' vector), as is the case in the rabbit (Barmack, J. Physiol., 1981, 314:547-564), and second, whether in the barbiturate anesthetized animal there was some difference in the way in which vertical canal primary afferents responded under the same two conditions. The gain of the VVOR differed between the two conditions and the dynamic response properties of some eighth nerve neurons innervating vertical canals also differed between the two conditions.

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334.11 ON EVALUATING DYNAMICS OF CENTRAL AND OCULAR RESPONSES IN THE VOR. H.L. Galiana and R.M. Douglas. Aviation Medical Research Unit, McGill University, Montreal, Canada H3G 1Y6.

Head rotation in the dark or the light produces, via the vestibulo-ocular reflex (VOR), ocular nystagmus consisting of slow compensatory and fast resetting eye movements. Assuming that each component of the eye movement represents the sampled response of separate slow and fast phase generators, estimation of slow-phase response dynamics currently involves 1) smoothing or averaging of firing rate in central responses, after removal of an eye position component (e.g. Lisberger & Miles, JNP 43:1725,80), or 2) differentiation of eye position and consideration of the resulting smoothed envelope of eye velocity. Such methods assume that the slow-phase segments of the observations represent pieces of a continuous steady-state response which is only intermittently available.

In fact, steady state conditions are never reached, as the same premotor cells in the vestibular nuclei (VN) participate in both the slow and fast phases of VOR responses (Nakao et al, EBR 45:371,82; Galiana & Outerbridge, JNP 51:210,84). Furthermore, the eye position component on some VN cell responses cannot be considered as an independent signal. Eye position is affected by premotor VN signals and its presence in the VN implies a feedback loop. Removal of this component in a VN cell response is equivalent to opening the feedback loop; the dynamics estimated from the remaining pattern of firing rate can thus be greatly in error from those of the complete system.

For these two reasons, a new approach to the analysis of VOR responses is required. A computer algorithm has been developed to extract dynamics from individual or groups of slow-phase response segments, be they neural or ocular. It relies on least-squares regression with respect to head velocity and eye position, allowing for variable initial conditions at the beginning of each slow phase segment. Theory predicts differences of as much as 90 deg in phase when using this technique, as compared to previous methods, especially in the case of central responses. Applications of the algorithm will be illustrated with simulated VN and ocular data; and with real VOR data recorded from alert cat.

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334.12 THE ROLE OF THE LOCUS COERULEUS IN THE GAIN REGULATION OF VESTIBULOSPINAL REFLEXES. O.Pompeiano, P.d'Ascanio and E.Bettini. Ist.di Fisiologia Um.,Catt.I,Univ.di Pisa,56100 Pisa,Italy.

Experiments of unit recording have shown that in decerebrate cats contraction of limb extensors during side-down roll tilt of the animal depends on both an increased discharge of excitatory vestibulospinal(VS)neurons and a reduced discharge of inhibitory reticulospinal(RS)neurons of the medulla;these neurons inhibit ipsilateral extensor motoneurons through Renshaw cells.Experiments were performed to find out whether the inhibitory RS neurons are under the tonic control of the locus coeruleus(LC)and,if so,whether this system intervenes in the gain regulation of VS reflexes.

In decerebrate cats electrolytic lesion limited to the LC of one side not only decreased the extensor tonus in the ipsilateral limbs,but greatly increased the gain(imp./sec/deg)of the multiunit EMG response of the corresponding triceps brachii to animal tilt(0.026 Hz,$\pm$10°).This finding did not depend on the decrease in postural activity following the lesion,since it was still obtained when an increased static stretch of the extensor muscle compensated for the reduced EMG activity.However,an increased postural activity and a reduced response gain of the muscle to labyrinth stimulation were elicited either by electrolytic lesion of the ipsilateral pontine tegmental region,from which the tegmento-reticular tract ending on the medullary inhibitory area originates,or by iv injection of atropine sulphate(0.25-0.50 mg/kg).It seems therefore that the inhibitory RS neurons of the medulla are tonically excited by a pontine tegmental system,functionally related to a cholinoceptive mechanism.We postulate that the higher the resting discharge of these pontine neurons and the related inhibitory RS neurons of the medulla,the greater would be the disinhibition of limb extensor motoneurons during side-down tilt,which increases the response gain of the corresponding muscle to labyrinth stimulation.The pontine tegmental neurons,however,are in turn inhibited by the tonic discharge of presumably catecholaminergic neurons located in the LC,which reduces the response gain of limb extensors to labyrinth stimulation.The LC system may thus operate as a variable gain regulator of the motor output during the VS reflexes.

334.13 VESTIBULO-OCULAR REFLEX GAIN CHANGES WITH CEREBELLAR LESIONS. RW Baloh*, JM Furman*, V Honrubia (SPON: M Brazier). Depts. of Neurology and Surgery (Head & Neck), UCLA School of Medicine, Los Angeles, CA 90024.

We studied the dynamics of the vestibulo-ocular reflex (VOR) in 11 patients with cerebellar atrophy who had increased amplitude of response on our standard impulse rotational test. Eye movements were induced by precise visual and vestibular stimuli, recorded with electro-oculography and analyzed on-line with a digital computer (Baloh RW et al., Aviat Space Environ Med 51: 563, 1980). Gain (peak slow phase eye velocity/peak chair velocity) and phase measured with sinusoidal rotation (0.0125 - 0.4 Hz) were compared with gain and time constants (time required for response to reach 37% of initial value) determined from velocity steps (acceleration 140°/s^2). Time constant values ranged from 1.3 to 16 seconds (mean $\pm$ 1 S.D., patients - 7.9 $\pm$ 5.1 sec, normals -12.2 $\pm$ 3.6 sec). Patients with short time constants had decreased gain at low frequencies of sinusoidal rotation while patients with long time constants had increased gain at low frequencies. All had increased gain at high frequencies. Phase lead values at low frequencies were inversely correlated with time constant values. All patients had profound impairment of pursuit, optokinetic nystagmus and fixation suppression of the VOR. Phase and time constants measured with fixation were approximately the same as those measured without fixation. There was no correlation between the degree of gaze-evoked nystagmus (with and without fixation) and time constant values. These findings are interpreted within the framework of a linear model of the central VOR that includes a feedback pathway through the cerebellum.

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334.14 EFFECTS OF ACTH-LIKE NEUROPEPTIDES ON VESTIBULAR COMPENSATION. U. Lüneburg* and H. Flohr* (SPON: C. Richter-Landsberg). Dept. of Neurobiology, University of Bremen, NW 2, 2800 Bremen 33, FRG.

Vestibular compensation, i.e., the process of functional recovery from the characteristic postural and locomotor symptoms caused by unilateral destruction of the labyrinth or vestibular nerve, is markedly influenced by ACTH-like peptides:

1. The acquisition of the compensated state can be accelerated by $ACTH_{4-10}$ (Met-Glu-His-Phe-Arg-Trp-Gly) in doses of 5-1000 µg/kg/day. The dose-response relationship depends on the degree of compensation.

2. The compensation process is slowed down significantly by hypophysectomy. The process so impaired can be restored to normal velocity by $ACTH_{4-10}$ (250 µg/kg/day).

3. The $ACTH_{4-9}$ analogue, Met (O_2)-Glu-His-Phe-D-Lys-Phe, accelerates the compensation process. This peptide is about 1000 times more effective than $ACTH_{4-10}$. High doses (5 µg/kg/day) are less effective, suggesting an inverted U-shaped dose-response curve.

4. (D-Phe^7)$ACTH_{4-10}$ (250-1000 µg/kg/day), which contains a dextrorotatory amino acid in position 7 of the $ACTH_{4-10}$ molecule, inhibits the compensation process; if given to partially or fully compensated animals it induces a decompensation, i.e., a temporary reappearance of the postural and locomotor deficits.

5. γ-MSH (Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly), which acts like (D-Phe^7)$ACTH_{4-10}$ in some learning paradigms, is without effect on vestibular compensation.

It is concluded that the pharmacological effects result from an interaction of the given peptides with endogenous peptidergic mechanisms that are physiologically involved in plastic processes.

334.15 DYNAMICS OF HEAD MOVEMENTS IN NORMAL HUMANS INDUCED BY WHOLE BODY OSCILLATIONS. M. Weinrich. Dept. of Neurology, Stanford University and Palo Alto VA Medical Center, Palo Alto CA 94304.

Vestibulocollic reflexes have been extensively studied in decerebrate animals. The significance of these reflexes in the control of normal head movements has been unclear. The dynamics of head oscillation induced by whole body oscillations of eight normal human subjects were studied. Subjects were seated on a velocity servo-controlled rate table and wore a lightweight helmet coupled to a rotary variable differential transformer which transduced head position. Head velocity was obtained by differentiating the head position signal electronically (bandpass .03-7 Hz.) Head velocity and table velocity were sampled at 100 Hz and stored on floopy discs for off-line analysis. Subjects were oscillated from .2 to 2 Hz. both with their eyes open and closed. At each stimulation frequency, table velocity and head velocity were averaged with ten trials each of five seconds duration. Data was analyzed by Fourier analysis and Bode plots of the gain and phase of head velocity with respect to chair velocity were computed.

In the frequency range .6-1.8 Hz. over 60% of the power in the head velocity was linearly related to chair velocity. The gain data can be accurately modeled with a purely passive model of the head and neck, but the phase lags observed are much larger than can be accounted for by a purely passive model. Gain and phase data can be fit by a model incorporating a vestibulocollic reflex which appears to differentiate the signal from the semi-circular canals. The gain and phase of head velocity are unaffected by the presence of vision.

Thus, the vestibulocollic reflex appears to substantially compensate for the pasive dynamics of the head and aid the VOR in stabilizing retinal images during body movements. Unlike the VOR, the vestibulocollic reflex appears unaffected by the presence or absence of vision.

This work was supported by a Veteran's Administration Medical Research Grant and the BRSG program at Stanford University.

334.16 VESTIBULAR STIMULATION VIA ARBITRARY OR RANDOM ROTATION FROM AN INEXPENSIVE SERVOMOTOR. R. S. Remmel and S. Doroodian, Biomedical Engineering Dept., Boston Univ., Boston, MA 02215 and R. Waldron, UAMS, Little Rock, Ark. 72205.

The vestibular system stabilizes the eyes over times as short as 10-20 msec (neuromuscular response) and as long as 10-30 sec (cupular time constant). For vestibular stimulation our servomotor responds in 0.1 sec to produce arbitrary motion under computer control with accelerations exceeding 200 deg/sec/sec and velocities exceeding 200 deg/sec. The frequency response is DC to 4 Hz with no load.

The 1/15 Hp Dayton gearmotor (type 2Z801A) operates at 115 V and costs < $100. Its 238:1 gear ratio produces 100 in.-oz. of torque for rotating small animals (other ratios are made).

The linear controller produces any speed between full forward and full reverse. Shaft position is fed back from a potentiometer. The controller is a transformerless pulse-width-modulated amplifier. Transistors switch first positive and then negative voltages to the motor at 5 KHz. The duty cycle for positive voltage compared to negative determines the average voltage to the motor.

Performance was measured with a white noise input. By cross-correlating the output with the input and averaging, using a PDP11/23 computer, we measured the impulse response, which consisted of a pure time delay of 0.05 sec and a pulse of about 0.1 sec width. Through use of a fast Fourier transform (FFT) on the output and on the input, the frequency response was measured. Response was flat to 4 Hz and then dropped off.

This white-noise technique may be useful for studying vestibular neural responses and eye movements. The motor is randomly rotated for several minutes while the computer digitizes signals. The system impulse response or, using the FFT, the Bode plot is obtained from this one section of data, a useful method when recording time is at a premium! The extension to nonlinear systems is called Weiner kernel analysis.

The controller and motor cost $350.

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SENSORY SYSTEM DEVELOPMENT II

335.1 METABOLIC CHANGES IN RAT INFERIOR COLLICULUS ASSOCIATED WITH EXPERIENTIAL FACTORS. W.J. Clerici* and J. Coleman. (SPON: J. Kosh). Dept. of Psychology, Univ. of South Carolina, Columbia, SC 29208

Previous work demonstrated a dorsolateral-to-ventromedial gradient in tonotopic organization in central nucleus of the rat inferior colliculus (CNIC). The present study examines metabolic changes along this continuum in 17 day old animals reared under two conditions and in normal adults. One group of animals was unilaterally ligated at 13 days and the occluded ear reopened on day 17. These animals were injected with 2-deoxy-D[1-^{3}H] glucose (200μCi/100 g.bw.) and stimulated through the previously deprived ear with a 50 kHz pure tone along with groups of normal 17 day old and adult rats. Other ligated 17 day old rats and 17 day old and young adult normals received no stimulation. Projected (coronal) autoradiographs were sampled for optical density at 5 points within each of 5 regions of CNIC bilaterally at 4 A-P locations.

Optical density in the ventromedial region representing high frequencies is higher than in the dorsolateral or intermediate areas in unstimulated young adults. With stimulation this regional difference becomes even greater on the side contralateral to ($p<.01$) and ipsilateral to ($p<.01$) the stimulated ear. However, the ventromedial area on the contralateral side is significantly more dense than that ipsilateral to stimulation.

Stimulation of nondeprived 17 day olds elicits enhanced activity in the contralateral ventromedial area as in adults. However, in stimulated deprived 17 day old rats a vertical band appears laterally in CNIC (oblique to the usual tonotopic bands) that is darker than the ventromedial area ($p<.01$). In nondeprived stimulated 17 day olds the optical density of the ventromedial region is higher than this lateral area ($p<.05$). Comparison of mean values between deprived and nondeprived 17 day olds indicates that ventromedial activity is not reduced due to deprivation, but that the lateral activity is elevated over normal. Inspection of autoradiographs from stimulated normal 13 day olds reveals similar lateral banding observed in deprived 17 day olds. With further maturation the ventromedial region becomes active in processing high frequencies and lateral activity is diminished. Monaural deprivation between postnatal days 13 and 17 possibly retards maturation of the tonotopic gradient. (Deafness Research Foundation.)

335.2 POSTNATAL REORGANIZATION OF THE CONNECTIONS OF THE TRIGEMINAL GANGLION. Tim O'Connor and Derek van der Kooy. Dept. of Anatomy, Univ. of Toronto, Toronto CANADA M5S 1A8

Trigeminal innervation of major cerebral arteries is responsible for migraine head pain. Cell bodies innervating the forehead and cerebral arteries are found in the same region in the ophthalmic branch of the trigeminal ganglion. These cells most likely converge on a common cell body in the trigeminal nucleus, thus resulting in referred head pain. However, an alternate interpretation of the referred pain is offered by the few trigeminal ganglion cells that have axon collaterals to the cerebral arteries and out to the skin of the forehead. In neonatal rats, many more ganglion cells with divergent axon collaterals are observed. For this reason we looked at how the pattern of cerebral artery innervation changed postnatally, resulting in the pattern seen in adults. True blue, (a retrograde fluorescent tracer that remains in labelled cell bodies for months without significant leakage), was applied to the middle cerebral artery, in postnatal (1-6 days) rats. Rats were sacrificed 5,10,22,54 and 90 days of age. Two days prior to sacrifice the frontal branch of the ophthalmic nerve was dissected free of tissue in selected rats at each survival time. Diamidino yellow was then applied to the cut nerve just proximal to the supraorbital foramen. The number and distribution of cell bodies in the ganglia were assessed for each stage postnatally. Rats sacrificed at days 5 and 10 had similar results. The average number of true blue fluorescent cells per ganglion was 663. Labelled cells were distributed throughout the ganglia, though the greatest number of cells were found in the ophthalmic branch. The number of labelled cell bodies decreased with increasing age. The number of true blue labelled cells at day 22,54, and 90 were 211,79 and 52 respectively. With increasing age the number of labelled cells in the mandibular and maxillary branches showed the greatest decrease with the result that most of the adult middle cerebral innervation is represented in the ophthalmic division. The development mechanisms responsible for this shift in representations are unknown. However initial results from the rats with the damidino yellow applications suggest that sensory neurons with collateral branches to blood vessels and the skin forehead region in the neonate, are the ones eliminated by adulthood and thus are partially responsible for the decrease in cell numbers. Perhaps these multibranched trigeminal neurons are at a competitive disadvantage during development.

335.3 WITHDRAWN

335.4 ORGANIZATION AND MODIFIABILITY OF THE CORTICAL LIMB REPRESENTATIONS. D.R.Dawson* and H.P.Killackey*. (SPON: F.Rice) Dept. of Psychobio., Univ. of Calif., Irvine, CA 92717.

Studies of rat somatosensory cortex have shown that each hemisphere contains a map of the entire contralateral body surface (C.Welker,J.C.N.'76). This somatotopic map is organized with the snout represented antero-laterally and the torso and hindlimbs represented postero-medially. Within this map, there is also a well defined representation of the forelimb and forepaw.

Rat pups (PND 7-10) were sacrificed under ether and perfused with 10% glycerol. The cortex was separated from the underlying white matter and flattened between glass slides. The tissue was quick frozen in isopentane, cut in a croystat, and reacted for succinic dehydrogenase(SDH).

We found a discrete anatomical organization in both the forepaw and hindpaw region of cortex. The forepaw area is composed of five elongated SDH bands oriented perpendicularly to the lip and face representation. Further, there are irregular subdivisions within each band. We suggest that each band corresponds to a peripheral digit. Between these bands and the face region, a hazy lightly-stained SDH crescent is discernable. This may be the physiologically defined dorsal paw region. Medial to the digit bands are five irregularly shaped SDH clusters which probably correspond to the palmar pads. A similar organization is found in the hindlimb area with the digit pads arranged parallel to each other and palmar pad clusters located more posteriorly.

The ventral surface of the hindpaw is primarily innervated by the sciatic nerve. The forearm is innervated by the median, radial, and ulnar nerves. Sectioning of these nerves on the day of birth results in a fusion of the SDH clusters in the associated cortical representation. Thus, our results suggest that the role of the periphery in organizing central representations previously hypothesized for the trigeminal system applies to other parts of the somatosensory system as well. (Supported by NSF grant #BNS81-20658 to H.P.K.)

335.5 FINE STRUCTURE IN VIBRISSAE-RELATED PORTIONS OF THE SPINAL TRIGEMINAL SUBNUCLEUS INTERPOLARIS IN NEONATAL RATS. L.S.Ide and H.P.Killackey*. Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

We have examined the fine structure of the rat's brainstem trigeminal complex on postnatal days 3 and 6 (PND 0 = day of birth) for comparison with that of the adult. In this study we selected the portion of subnucleus interpolaris that, in the neonate, showed vibrissae-related segmentation patterns using cytochrome oxidase histochemistry. At the electron microscopic level the cytochrome oxidase-rich segments of this pattern are regions which have high concentrations of reactive mitochondria within neuronal somata and dendrites.

In the adult rat the subnucleus interpolaris is characterized by a population of neuronal somata that vary widely in size (7-40um), shape, and dendritic distribution. By contrast, cells in the neonate are generally small (5-12um) and more uniform in appearance. Dendrites emerge predominantly from one pole of the soma, and a profusion of cytoplasmic organelles is associated with that pole. These developing neurons are more densely aggregated than in the adult.

The neuropil in the neonate contains numerous fine unmyelinated axonal processes with a few larger axons beginning to acquire myelin sheaths on PND 6. Precursors of two types of mature myelinated axon bundles are evident: "rays," small bundles penetrating the neuropil at right angles to the trigeminal tract, and "deep axon bundles," large axon bundles oriented rostrocaudally (see Gobel & Purvis,'72).

In the neonate, synaptic terminals are less numerous than in the mature nucleus and smaller in size. Further, synaptic contacts are largely restricted to small dendrites and other fine neuronal processes in the neuropil. Axosomatic contacts (characteristic of larger neurons in the adult) are rarely seen in neonates. A subset of synaptic terminals seen on PND 3 make multiple synaptic contacts onto small arrays of dendrites. These arrays are more complex on PND 6 with a few examples of axoaxonic synaptic contacts seen in association with them. Such arrays appear to be precursors of the synaptic glomeruli characteristic of the adult subnucleus interpolaris.

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335.6 INFRAORBITAL NERVE SECTION IN NEWBORN RAT ALTERS THE TERMINAL ARBORS OF THALAMOCORTICAL AXONS PROJECTING TO SOMATOSENSORY CORTEX. K. F. Jensen and H. P. Killackey*. Dept. of Psychobiology, Univ. Calif., Irvine, CA 92717.

The pattern of thalamocortical projections to the barrel field of the rat somatosensory cortex reflects the spatial arrangement of receptors associated with the sinus hairs and vibrissae of the face. We have previously demonstrated that horseradish peroxidase (HRP) injected beneath the cortical barrel field labels axons having terminals in layers III, IV, and VI, a pattern which corresponds to the terminal degeneration seen after lesions of the ventrobasal thalamus. These presumed thalamocortical axons branch extensively in layer IV and have terminal fields that correspond in size and arrangement to cortical barrels. The terminal field of a single axon tends to fill the center of a barrel. Axons projecting to the same barrel have largely coexstensive terminal fields. In the present investigation we have examined the effect of neonatal nerve cut on the morphology of the terminal arborizations of these presumed thalamocortical axons.

The infraorbital nerve, which innervates the vibrissae pad, was cut on the right side of the face in newborn rats. When the animals reached 30-90 days of age, 5% HRP-WGA (Sigma) was injected bilaterally into the white matter or caudate-putamen. The labeled axons were visualized by the DAB-GOD method of Itoh et. al. '79, (Brain Res. 175:341).

Labeled axons in the left hemisphere appear to have fewer branches and a lower density of terminals than axons in the right hemisphere. The arborizations in layer IV are also not as profuse. In the most extreme cases, axons branch evenly throughout layers III through VI and lack a dense concentration of terminals in layer IV. When fibers within the same area of cortex are labeled they can exhibit partially overlapping terminal fields. Thus, neonatal nerve cut alters the branching pattern and the distribution of terminals of thalamocortical axons.

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335.7 TOPOGRAPHIC REPRESENTATION OF VIBRISSAE FOLLICLES IN THE TRIGEMINAL (V) GANGLION OF NORMAL ADULT RATS AND ADULTS SUBJECTED TO NEONATAL TRANSECTION OF THE INFRAORBITAL (IO) NERVE. M. Math*, B.G. Klein and R.W. Rhoades (SPON: H.P. Zeigler) Dept. of Anatomy, University of Medicine and Dentistry of N.J., School of Osteopathic Med. and Rutgers Med. School, Piscataway, N.J. 08854.

It is well known that damage to the whisker follicles or IO nerve in neonatal rodents alters the central anatomical correlates of the vibrissae. Less attention has been paid to the organization of the whisker representation in the V ganglion and the way in which it may be changed by such damage. In this study, we have used retrograde transport of wheatgerm-agglutinin horseradish peroxidase and fluorescent tracers (true blue, fast blue, nuclear yellow and diaminido yellow) to delineate the organization of the whisker representation in the V ganglion of both normals and animals subjected to IO nerve transection within 12 hr of birth.

Injections of different tracers into follicles of A, C and E row in normals or the unoperated side of lesioned rats revealed a clearcut topography. Cells innervating E-row were most numerous in the lateral part of the ophthalmic-maxillary portion of the ganglion and those labelled by A-row injections were most numerous medially. Cells projecting to C-row were, for the most part, interposed. Overlap between A and C row or C and E row ganglion cells was common, but it was observed much less often for A and E row cells. Injections of different tracers into A1 and A4 or C1 and C5 provided no clear evidence for an ordered ganglionic representation of the rostrocaudal axis of the whiskerpad.

Injections on the operated side in neonatally lesioned rats labelled many fewer ganglion cells than in normals. Topography was also less clear than that in the normal ganglion. In most cases, the relative distributions of ganglion cells innervating different rows were maintained. However, interdigitation of A and E row cells was very common and, in some animals, distributions of neurons innervating these two rows overlapped completely. As in normals, there was no clear evidence of a topographic representation of the rostrocaudal axis of whiskerpad.

None of our cases provided evidence that ganglion cells innervated multiple follicles. However, to demonstrate topography, widely separated follicles were injected.

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335.8 **CHANGES IN THE PATTERN OF TRIGEMINAL PRIMARY AFFERENT TERMINATIONS IN THE BRAINSTEM OF THE RAT AFTER PERIPHERAL MANIPULATIONS.** C.A.Bates and H.P.Killackey.* Dept. Psychobiol., UCI, Irvine, CA. 92717

In the trigeminal complex of the rat, clusters of SDH activity replicate the pattern of rows of vibrissae on the face. Removal of a row results in a decrease in SDH activity within the associated brainstem area. We sought to determine whether the change in SDH activity reflects a decrease in the activity of or an actual loss of afferent projections

The vibrissae of rows B and D or row C alone were removed and the follicles cauterized on the day of birth (PND 0). On PND 5, the trigeminal ganglion was injected with 2-5 microliters of HRP (4% WGA-HRP, 10% Sigma VI). Animals were sacrificed on PND 7 and processed with the method of Itoh et al. (Br. Res. 175:341, 1979).

Within the principle sensory nucleus, there is dense terminal labeling throughout and rows of clusters can be discerned in the caudal-ventral portion of the nucleus. In cases with vibrissae removed at birth, bands of less dense terminal labeling are observed in areas which correspond to the damaged rows. Further caudally, in subnuclei interpolaris and caudalis the clusters of terminal arborizations associated with the normal pattern of HRP labeling are absent in areas related to the damaged rows of vibrissae, although they are still present in the areas associated with non-damaged rows.

Thus, within each of these representations, there is a decrease in the density of HRP labeled fibers and terminals corresponding to the damaged rows of vibrissae. Although it is possible that the fibers of the damaged rows are still present in the brainstem, but unable to transport HRP, we interpret the data as demonstrating a loss of trigeminal afferents in the affected areas of the vibrissae representation.

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335.9 A QUANTITATIVE ELECTRON MICROSCOPIC ANALYSIS OF THE INFRAORBITAL NERVE IN THE NEWBORN RAT. A.M. Szczepanik*, W.E. Renehan* and R.W. Rhoades (SPON: G. Krauthamer) Dept. of Anatomy, Univ. of Medicine and Dentistry of New Jersey, School of Osteopathic Medicine and Rutgers Medical School, Piscataway, N.J. 08854.

The infraorbital (IO) nerve, a branch of the maxillary division of the trigeminal (V) nerve, provides the sole afferent innervation of the rodent mystacial vibrissae. The central representations of these whiskers have become an important model for the study of central nervous system development and plasticity, but, surprisingly, relatively little is known regarding the composition of the nerve itself. We (Jacquin et al. Brain Res. 290:131-135, 1984) have previously shown that the IO nerve in adult rats is composed of 19,740 (sd.=2,054) myelinated and 13,319 (sd.= 1,159) unmyelinated axons. In the present study we have used electron microscopic methods to delineate the organization of this V branch on the day of birth, an age at which damage to the nerve has been induced in numerous studies.

Neonatal rats (N=3) were perfused through the ascending aorta with mixed aldehydes within 6 hr of birth. The IO nerve was then dissected and prepared for electron-microscopy using standard techniques. Electron micrographs were taken at a magnification of 1,250 X and printed at a final magnification of 8,000 X. Complete montages of the nerve were constructed and every fiber was counted. These counts were rechecked using higher magnification (16,500 X) prints from the same nerves. The latter prints were also employed for measurements of fiber size.

The IO nerve in the newborn rat contained an average of 42,051 (sd.=2,083) unmyelinated and 168 (sd.=47) myelinated axons. The average diameter for the unmyelinated axons (N=1,581) was 0.46 μm (sd.=0.16) and that for the myelinated fibers (N=248; measurements include myelin sheath) was 1.71 μm (sd.=17).

These data demonstrate that the IO nerve in rat contains approximately 10,000 more fibers on the day of birth than it does in adulthood and further that the proportion of myelinated to unmyelinated axons at this age is quite immature. Results from another study (Davies, A. and Lumsden, A. J. Comp. Neurol. 223:124-137, 1984) suggest that the reduction in IO fiber number may result from ganglion cell death rather than retraction of supernumary axons.

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335.10 CHANGES IN NUMBERS OF DORSAL ROOT AXONS DURING DEVELOPMENT. R.E. Coggeshall, K. Chung and C.E. Hulsebosch. Marine Biomed. Inst., Anat. and Physiol. and Biophys., Univ. of Tex. Med. Branch, Galveston, TX 77550.

An important principle of neural development is that a surplus of neurons arise in early development and then the excess die to produce the definitive adult numbers. By contrast to our understanding of cell numbers, however, we know little about changes in axonal numbers during development and since axons are the communicating channels of the neurons, it is also important to determine how their numbers change. In the present study, we have counted all axons in the T5 and S2 dorsal roots of the post-natal rat and the data we have gathered are presented below.

	1 week	10 days	2 weeks	1 month	Adult
T5	6192	----	----	5690	5839
S2	----	4281	3765	3367	2797

In both cases, it can be seen that young animals (1 week and 10 days) possess more axons in their dorsal roots than the older animals and that, particularly in the sacral roots, there seems to be a steadily diminishing number of axons as the animal ages. If we assume that the definitive number of dorsal root ganglion cells is determined before birth, and if we also assume that further data support these preliminary observations on axonal numbers, then axonal numbers are changing long after the cells that give rise to the axons have stabilized. Thus it would seem that factors that determine the numbers of axons are different, at least in a temporal sense, from those that determine the numbers of cells. It is our opinion that the factors that determine the numbers of adult axons are as important as those that determine the number of adult neurons.

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335.11 THE ENHANCED NEURAL RESPONSE INDUCED BY POSTNATAL OLFACTORY EXPERIENCE IN NORWAY RATS IS ODOR-SPECIFIC. R. M. Coopersmith* and M. Leon. Department of Psychobiology, University of California, Irvine CA 92717.

Norway rat pups develop a preference for the odor of their mother, or an arbritrarily selected odor, based on postnatal experience. Daily exposure to peppermint odor during the first 18 days of life will induce both an attraction and an increased neuronal response in specific olfactory bulb glomeruli to peppermint odor. Since it is possible that experience with one odor enhances the glomerular response to any odor, we gave pups experience with cyclohexanone odor and then tested them on day 19 with peppermint odor.

On days 1 to 18 after birth, rat pups received exposure to either peppermint odor (peppermint-familiar) or cyclohexanone odor (cyclohexanone-familiar) from a flow-dilution olfactometer. On day 19, all pups were injected with ^{14}C-2-deoxyglucose (200 uCi/kg) and given a 45 min test exposure to peppermint in an apparatus that allowed us to analyze respiration rate and sniff frequency. We then prepared autoradiographs of olfactory bulb sections along with calibrated standards. The films were then analyzed with a computer-based image processing system which allowed quantitative comparisons between uptake sites to be made.

Peppermint-familiar pups showed enhanced activity in three laterally-located complexes of glomeruli, 1.5-2.2 mm from the rostral pole of the bulb, confirming our previous findings. The same peppermint test exposure given to cyclohexanone-familiar pups induced significantly less activity in these same glomerular areas.

The enhanced neural activity in the peppermint-familiar animals was not a result of an increased odor stimulus to the olfactory system of these pups. The overall number of respirations for both groups of pups were not different during the test, and the number of high frequency respirations characteristic of sniffing were virtually identical. We have shown that early exposure to an odor will induce an enhanced olfactory bulb response specific to that odor.

335.12 RECEPTIVE FIELD SIZE OF PERIPHERAL TASTE FIBERS IN FETAL AND PERINATAL SHEEP. C.M.Mistretta and R.M.Bradley. Dept. Oral Biology, Univ. Michigan Sch. of Dentistry, Ann Arbor, MI 48109.

Summated responses to chemical stimuli recorded from the chorda tympani nerve change during development in sheep fetuses, lambs and adults. In young fetuses NaCl elicits small magnitude responses and NH_4Cl elicits large responses. In adults responses to both of these salts are of very large magnitude. Changing chemical responses might relate to developmental differences in the number of fungiform papillae and taste buds innervated by single chorda tympani fibers. Therefore we have mapped receptive fields in two age groups: fetuses aged 126-130 days of gestation (term = 147 days) and perinatal animals aged 145 days of gestation to 7 days postnatal. Single chorda tympani fibers were dissected, responses to 0.5M NH_4Cl and NaCl were recorded, and the general location of the receptive field was determined. Then the number and location of papillae innervated by the fiber were determined by electrically stimulating single fungiform papillae with 3-5 μamp anodal current. Thus far six single units have been studied in 5 fetuses and 10 units in 7 perinatal animals.

None of the fetal units responded to NaCl, but each of them responded to NH_4Cl. In contrast, all perinatal units responded to NaCl and NH_4Cl. The mean receptive field size of fetal units was 7.2 papillae (S.D. ± 3.2), with a range of 1 to 10. Perinatal units had a mean field size of 10.3 papillae (S.D. ± 6.5), with a range of 2 to 20. The difference in field size is not significant.

Although the likelihood of obtaining a response to NaCl is greater in units from perinatal animals than fetuses, our preliminary data indicate that the receptive field size of taste units is not different in these two age groups. However, since the number of taste buds increases during development, the same number of papillae in receptive fields in perinatal animals might contain more taste buds than in fetuses. Papillae from each receptive field that has been mapped are being examined to determine the number of taste buds per field. Furthermore, many more receptive fields must be mapped and additional age groups studied before concluding that there is no developmental difference in the number of papillae innervated by a single chorda tympani fiber.

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335.13 DEVELOPMENT OF CONTRAST SENSITIVITY IN INFANT MACAQUE MONKEYS. R.G.Boothe, Infant Primate Lab, WJ-10, University of Washington, Seattle,Wa 98195

Seven infant macaque monkeys were trained to make operant discriminations in a face mask testing cage. The infants were trained and then tested with a two-alternative forced-choice psychophysical procedure. The infants discriminated spatial sinusoidally modulated gratings from homogeneous fields matched for mean luminance. All testing was bincoular with natural pupils.

Quantitative examinations of the psychometric functions obtained from these infants reveal significant improvements in contrast sensitivity as a function of age, but no significant changes in the slopes of the psychometric functions.

The time courses over which contrast sensitivity improves is not the same for all spatial frequencies, and this leads to developmental changes in shape of the contrast sensitivity function. Cutoff frequencies improve from an average of 4.5 cy/deg at 5 weeks of age to an adult asymptote of 40 to 50 cy/deg by one year of age. Peak sensitivity (1/threshold contrast) increases from 18 at 5 weeks better than 100 by one year.

335.14 VISUAL FUNCTION IN GOLDFISH FOLLOWING BILATERAL TECTAL ABLATION. B.E. Schlumpf* and R.E. Davis. (SPON: D.G. Green). Univ. of Michigan, Neurosci. Lab., Ann Arbor, MI 48109

More than 90% of goldfish retinal ganglion cell fibers project to the optic tectum. This structure is necessary for a number of behavioral responses including food pellet localization, shadow induced deceleration of respiration and the optomotor response (Springer, A.D., Easter, S.S & Agranoff, B.W., Brain Res., 128:393, 1977). The behavioral function of retinal projections to nontectal areas, including preoptic, thalamic, hypothalamic and pretectal nuclei (Springer, A.D. & Gaffney, J.S., J. Comp. Neurol., 203:401, 1981) is poorly understood. We investigated vision in tectum-ablated goldfish (BOT) using a behavioral method. Visual responding was assessed by measuring the occurrence of a branchial suppression response to a visual stimulus (CS) that was classically conditioned to an electric shock stimulus (US). Bilateral optic tectum ablation blocked response to the CS in the presence of overhead illumination but not in darkness. BOT fish tested in darkness (N=6) responded to the CS when initially tested one week after surgery. Optic nerve crush blocked responding indicating that the response is retinally, rather than extraretinally mediated. Recovery of response occurred 2-3 weeks postaxotomy (WPA) which is similar to the post crush recovery time in intact-tectum fish (Davis, R.E., Schlumpf, B.E. & Klinger, P.D. Neurosci. Abstr. 10, 1984). Additional BOT fish (N=11) were tested in darkness to measure their sensitivity to the CS. During the first six weeks following tectal ablation, the percent of fish responding increased from 45-100% and individuals showed increased sensitivity. Fish tested in the presence of overhead illumination (N=4) failed to show recovery of response when tested up to 20 WPA. These results indicate that normal nontectal retinal projections can mediate behavioral responding to a visual CS. The increase in sensitivity and percentage of fish responding may be owed to an increase in innervation of nontectal visual areas following regeneration (Yager, D., Sharma, S.C. & Grover, B.G., Brain Res., 137:267, 1977). It may also reflect increased conditioning of visual input to nontectal visual areas. Another possibility is that regenerated axons innervate nonvisual brain areas (Sharma, S.C., Nature, 291:66, 1981) which can mediate this behavioral response.

335.15 ANATOMIC AND PHYSIOLOGIC DEVELOPMENT OF THE PIGEON RETINO-TECTAL SYSTEM. P.Bagnoli*,V.Porciatti*,A.Lanfranchi* and C. Bedini*. (SPON A. Cangiano).Inst.of Physiology,University and Inst.of Neurophysiology,C.N.R.,via S.Zeno 31-51,Inst.of Biology,via Volta 6,56100 Pisa,Dept.Ophthalmology,U.S.L.13 Livorno,Italy.

In the present study, the development of light evoked activity in the pigeon retino-tectal system was investigated and compared whith the structural maturation of retinal cells during the first 20 days post-hatching. The first ERG (either to flash or pattern-reversal stimulation) can be recorded at 4-6 days simultaneously to the appearance of photosensitive lamellae in the retinal outer segments. At the same time only few synapses were present in the OPL and they increased in number as the lamellar structures completed their maturation. Flash and pattern ERG's increased in amplitude during the first 20 days when adult waveforms can be recorded. The similar onset and amplitude maturation of flash and pattern ERG's suggest similar intraretinal generators for both kind of responses. This hypothesis is in agreement with previous studies which suggest that the generators of the pigeon pattern-ERG might be the same of the flash evoked b-wave (Holden and Vaegan,Vision Res.,23:561,1983; Bagnoli et al.,Exp. Brain Res.,54:1,1984). Evoked potentials to patterned and unpatterned stimuli can be recorded from the tectal surface at around 8 days post-hatching. Retinal and tectal acuities increased in parallel of about 1.5 octaves, to reach 4-5 cycles deg^{-1}, at around 20 days. This value corresponds to the psychophysical acuity of adult pigeons for the lateral visual field. Preliminary experiments in long term monocularly deprived pigeons (Burkhalter and Knüsel unpublished)reported acuity deficits of the deprived eyes. A further aim of this study was to establish whether this effect might be attributed to a reduction of acuity in the retino-tectal system. Our results demonstrate that retinal and tectal responses to patterned stimuli were not impaired by monocular deprivation. We can conclude that behavioural loss in visual acuity does reflect a damage in visual relays central to the retino-tectal pathway.

335.16 MISALIGNMENT OF AUDITORY AND VISUAL MAPS IN THE OPTIC TECTUM MATCHES SOUND LOCALIZATION ERRORS IN BARN OWLS. E.I. Knudsen and P.F. Knudsen. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA.

Sound localization behavior is altered in barn owls that have had one ear plugged before the eighth week post-hatching (Knudsen et al., J. Neurosci. 4: (4), 1984). When the earplug is removed from these animals they make large localization errors. If the earplug is removed before the end of a critical period, the owl gradually corrects the error; if the earplug is removed after the critical period, the owl does not correct its error.

The representation of auditory space in the optic tectum also is altered by early monaural plugging (Knudsen, Science, 222: 939, 1983). In adult animals that have been plugged since 5 weeks of age, single units have auditory best areas that align with their visual receptive fields when the earplug is in place. When the earplug is removed, auditory best areas become misaligned with respect to visual receptive fields.

In experiments reported here, behavioral and neurophysiological experiments were conducted to compare sound localization errors with field misalignment of tectal units in the same animal. The measure of sound localization was a reinforced head orientation to a noise source. Auditory best areas were measured using a movable, free field noise source. Visual receptive fields were plotted on a hemispheric screen. In three owls, localization errors following earplug removal were equal in magnitude and direction to the misalignment of auditory and visual fields of tectal units with frontal receptive fields. Two of these animals were unplugged before the end of the critical period and their localization errors and receptive field misalignments decreased at comparable rates over the next few weeks. The third animal was plugged past the end of the critical period and both its localization error and the misalignment of auditory and visual receptive fields in the tectum remained constant for at least 6 months after the removal of the earplug.

The agreement between the behavior and physiology suggests that auditory-visual misalignment measured in the tectum offers a reliable neural correlate of the experience dependent alterations of sound localization that have been documented behaviorally.

335.17 RHYTHMIC SPONTANEOUS ACTIVITY IN THE DEVELOPING AVIAN AUDITORY SYSTEM. W. Lippe. Dept. of Otorhinolaryngology, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73126.

Microelectrode recordings of multiunit activity were made in nucleus magnocellularis (NM) and nucleus laminaris (NL), first and second order nuclei in the avian auditory system. The pattern of spontaneous activity in 17 day chick embryos was compared with that previously observed in hatchlings. Embryos were supported in a humidity and temperature (37.5°C) controlled chamber inside a sound attenuated room. Their scalp was infiltrated with a local anesthetic and spontaneous movements were eliminated with intramuscular injections of Flaxedil.

Multiunit recordings show that spontaneous activity in NM and NL of hatchlings does not occur with any obvious pattern. In contrast, spontaneous activity in embryos occurs in bursts at regular two second intervals. This pattern of rhythmic discharge has not been observed in recordings from nonauditory regions of the brain stem. Preliminary observations show that the interval between bursts of spontaneous activity changes during development, being longer in 16 day embryos (2.5 seconds) and shorter in 18 day embryos (1.7 seconds). The rhythmicity can be blocked, enhanced or reset in a predictable manner by sound stimulation. It is abolished by cochlear removal. Control observations have shown that the rhythmic pattern is not due to movements of the embryo, middle ear muscle contractions or the embryo's heart beat. Bursts occur bilaterally but not simultaneously on the two sides of the brain. Thus, the rhythmicity is not caused by a general systemic factor. It is also unlikely that the rhythmicity is associated with a deteriorated condition of the embryo. When rhythmic discharges are present the embryo remains in excellent condition; whereas, if the rhythmicity becomes poorly defined or disappears, the embryo dies within an hour.

It is concluded that a rhythmic pattern of spontaneous activity occurs in NM and NL during embryonic development but not after hatching. It's mode of generation and functional significance for auditory system development remain to be determined. (The initial observations of this phenomenon were made while the author was in the Dept. of Otolaryngology at the Univ. of Virginia Medical Center and was supported by NIH grant NS15478 and RCDA NS00305 to E.W Rubel. Work presently supported by Univ. of Oklahoma College of Medicine Award C1177501 and NIH grant NS20724 to W. Lippe.)

335.18 PALMITATE UPTAKE IN VISUAL AND AUDITORY NEURAL PATHWAYS AFTER UNILATERAL SENSORY DEPRIVATION. A.S. Kimes, J.C. Miller*, J.M. Bell*, and S.I. Rapoport. B.C.H. Baltimore, MD 21224 and NIA, NIH, Bethesda, MD. 20205.

Palmitate uptake is proportional to $rCMR_{glc}$ and blood flow in the awake resting rat brain, is reduced by pentobarbital anesthesia and not affected by increased blood flow. The present study addresses the effects of unilateral deprivation on the palmitate uptake as measured by the method of Kimes et al. (Brain Res. 274:291, 1983) on the visual and auditory systems. The uptake of (14C)palmitate into a stable compartment of the brain at 4 h was studied in well-fed, adult male, Osborne-Mendel rats which were subjected to one of the following three treatments: 1) unilateral enucleation (n=4), 2) unilateral ear occlusion (n=4) or 3) no treatment (n=6). Femoral arterial and venous catheters were implanted under pentobarbital anesthesia. Enucleation or occlusion was preformed while the rat was maintained under the anesthesia. Rats were allowed 4 h to recover from anesthesia, following which 450 μCi/Kg (14C)palmitate was injected i.v., and timed arterial blood samples were collected. At 4 h after injection, rats were killed and brains were frozen. Brain radioactivity was determined in 40 regions including those which are associated with the visual and auditory pathways by quantitative autoradiography. Plasma radioactivity due to (14C)palmitate was determined by extraction and thin layer chromatography. Unesterified plasma palmitate was determined by gas chromatography. Palmitate uptake, $rCMR_{palm}$, into the stable brain was calculated from the equation $rCMR_{palm} = k\ Cplasma$, where Cplasma = plasma concentration of palmitate and k, the transfer constant for plasma palmitate to brain, $= C^*brain / \int_0^4 h\ C^*plasma\ dt$, where C*brain = the regional brain radioactivity at 4 h and $\int_0^4 h\ C^*plasma\ dt$ = integral of plasma (14C)palmitate between 0 and 4 h. There were no right - left differences in $rCMR_{palm}$ or k in any brain structures including those normally associated with visual and auditory sensory pathways. Regional $rCMR_{palm}$'s and k's of either treatment were not different from controls. $rCMR_{glc}$ was measured under the same experimental conditions described above and the expected decrements in glucose utilization were found unilaterally in the auditory and visual structures. The present study indicates that an acute sensory deprivation does not cause a decrease in palmitate uptake in the neural pathways of the affected sensory system.

335.19 AChE REACTIVITY IN IMMATURE RAT THALAMUS: DYNAMIC PROPERTIES AND ONSET IN RELATION TO MIGRATION. R.A. McGowan* and D.A. Kristt (SPON: S. Freimark). Neuropath. Service, Stanford Univ. School of Med., Stanford, CA 94305.

In view of the ultrastructural evidence (Kristt, D.A., Neurosci., 10:923, 1983) suggesting that immature non-cholinergic VB neurons transiently synthesize AChE, several additional questions arise that bear on the issue of whether this synthetic capability is vestigial or functional. First, is AChE reactivity responsive to extrinsic influences, i.e., can it be altered by experimental manipulation? This question was investigated using pharmacohistochemical and surgical strategies. It was found that (i) 18-24 hours following **anti-cholinesterase (DFP) treatment,** VB neurons in 6-8 dpn rats are equivalent to untreated pups in their high levels of reactivity; (ii) at 2 hours post-treatment AChE is almost completely inhibited; and (iii) unilateral **vibrissal pad denervation** alters reactivity in ipsilateral VB. VB on the side contralateral to the lesion--receiving the trigeminal input from the denervated vibrissae--is quite typical of 6-7 dpn VB and shows a normal differential in staining between VBm and VBl with VBm being less intensely stained than VBl. However, an abnormal focal reduction in staining intensity was seen in the area of VBm corresponding to the vibrissal representation. There is no evidence of atrophy. In the ipsilateral VB, the nucleus is uniformly darkly stained so that VBm cannot be distinguished from VBl. This indicates an absolute increase in AChE reactivity in ipsilateral VBm. **Conclusions:** 1) The AChE synthesized by immature VB neurons is dynamically regulated during the period of transient reactivity; and 2) the vibrissal lesions suggest a relationship between AChE reactivity and afferent input.

Another question examined was how the time course for the genesis of VB neurons (15 days post conception, dpc) and migration compares to the time of onset of AChE staining in VB neurons. AChE positivity has been noted previously in some primitive neuroectodermal cells and neuroblasts during this period of neurogenesis and subsequent migration. A role for AChE in these processes has therefore been postulated. Light microscopic observations suggest that at 16-17 dpc the ventral thalamus is identifiable cytoarchitectonically. This suggests that substantial migration of VB neurons to the ventro-posterior aspect of the thalamus is likely to have occurred by this date. No staining can be observed until 18-19 dpc gestation (Birth: ca. 21 dpc). It can be tentatively **concluded** that AChE begins to be synthesized in substantial concentrations following the period of neurogenesis and migration, i.e. in the early phases of cell differentation and connectivity formation. Support: NSF Grant BNS 81-40895.

335.20 DIFFERENTIATION AND GROWTH OF NEUROFILAMENT CONTAINING NEURONES IN NORMAL RATS AND AFTER INTRAOCULAR TRANSPLANTATION. C. Ayer-Le Lievre*, D. Dahl, A. Bignami, H. Björklund* and A. Seiger. Dept. of Histology, Karolinska Inst., Stockholm, S-10401 and Spinal Cord Injury Res. Lab., West Roxbury VAMC and Harvard Med. School, Boston, MA 02132.

Immunoreactivity to neurofilament (NF) antiserum first appears in 10 somites (10 days) rat embryos in the ventromedial part of anterior rhombencephalic neural tube. NF-positive cell bodies appear one day later in the peripheral nervous system. In 5 mm (11 days) rat embryos, the NF-immunoreactive material only forms a ring around the nuclei of cranial sensory ganglionic neurones, while in the corresponding central motor areas of the neural tube, cell bodies are filled with immunoreactive material. After 16 days, while differentiating, some peripheral sensory neurones become loaded with an eccentric meshwork of NF. Such neurones are found in trigeminal, petrous, nodose and dorsal root ganglia. In some autonomic ganglion cells NF material forms a perinuclear ring. Intensely reactive fibres are present in sensory ganglia and nerves, in autonomic ganglia and, to a lesser extent, in autonomic nerves. By the end of the gestation period, the distribution of NF-immunoreactivity mimicks that observed in adults.

In whole mounts of adult rat irides, a dense plexus of NF-positive fibres and prominent bundles are observed. These fibres originate in the trigeminal ganglion. When adult irides are transplanted to the anterior eye chamber of adult recipients, this plexus disappears during the following 4 days. However, by the end of this period, a few regrowing NF-positive fibres from the host iris start reinnervating the graft. This extrinsic innervation progressively extends into the grafted iris. After 3.5 months the density of the newly formed plexus is similar to that of normal irides but its pattern is different; the NF-positive fibres of the grafted iris are mainly thin and rather oganized into a network of individual or paired fibres.

The growth capacity of NF-positive nerves from the trigeminal ganglion was studied by grafting fetal trigeminal ganglia into the eye chamber of adult rats. A dense halo of radiating fibres rapidly formed around the ganglion into the host iris. To eliminate intrinsic fibres of the host, such irides and graft were themselves transplanted into the eye chamber of new recipients for 3 to 4 days. In these condition NF-positive fibres originating from the ganglion could be observed in the first host iris at long distances from the graft. Supported by Swedish MRC, French CNRS and VA.

335.21 POLYSENSORY EVOKED POTENTIALS IN VISUAL AND PREFRONTAL AREAS IN THE DEVELOPING RAT IN RELATION TO BEHAVIORAL STATES. Guenther H.Rose* and Majid Mirmiran*, Dept. of Psychology, Boudoin College, Brunswick, Maine 04011 (U.S.A.), and Netherlands Institute for Brain Research, Amsterdam 1095 KN (The Netherlands) (SPON:ENA).

The influence of modality of stimulation (15 µsec flash; 10 ms, 1000 - 6000 Hz tone), recording sites (cortical areas 17 and 8), and behavioral state (wakefulness, quiet sleep, and active sleep) assessed by EEG and EMG recordings, on the occurrence, wave form, and latency of cortical evoked potentials, were assessed in immature (10-20 days of age), juvenile (25-30 days of age), and adult rats. Beginning with a long latency (150-160 ms) negative monopolar response to flash in area 17 at 10 days of age, a characteristic sequence of waveform, amplitude and latency changes occurred as a function of age and behavioral state, with a mature-like waveform rapidly achieved by 16 - 18 days of life; latency continued to decrease until day 28. Responses to tone in area 17, as well as to light and tone in area 8, which also began at 10 days of age, tended to be more variable and were usually of larger latency (5 - 10 ms).

The amplitude of the cortical evoked response was highest and the latency was shortest during alert and drowsy states of the wakefulness. Upon transition to quiet sleep the latency of response was increased, while during REM-sleep it was identical to that in an alert state.

335.22 SURGICALLY DISPLACED nVIII AND nVII FOLLOW NORMAL CNS PATHWAYS TO AN IDENTIFIED TARGET NEURON. W.S. David and P.G. Model. Dept. Neurosci., Albert Einstein Coll.Med., Bronx, NY 10461

There is a precise ordering of synaptic connections in the vertebrate CNS. In the amphibian Mauthner cell (M-cell) system, the vestibular (nVIII) and lateral line (nVII) nerves form major sources of sensory input to the ipsilateral M-cell. In the axolotl (Ambystoma mexicanum), nVIII synapses are restricted to the ventral surface and branches of the M-cell's lateral dendrite and nVII terminals are confined to the dendrite's dorsolateral branches. Previous studies have shown that displacement of the developing ear and associated nVIII and nVII ganglia along the anteroposterior axis, prior to nerve outgrowth, does not alter the subsequent patterning of connections on the M-cell (David and Model, 1982, Anat. Rec., 202: 41a; David and Model, 1982, Soc. Neurosci. Abstr. 8: 436). The present study defines the pathways of the ectopic nerves.

Horseradish peroxidase (HRP)-labeling of a control nVIII in a 21mm feeding larva revealed a discrete rostrocaudally oriented tract located in the ventrolateral white matter. Labeling of a control nVII demonstrated three anteroposterior running tracts in the dorsolateral neuropil. The location of these nVIII and nVII tracts was similar to that described by Herrick (1914, J.Comp.Neur. 203: 343). Prospective ear and associated nVIII and nVII ganglia were unilaterally transplanted rostral or caudal to their usual position in the embryo. The displaced nVIII and nVII were labeled with HRP when the animals had reached 21mm larval stages. LM analysis revealed transplanted ears and ipsilateral M-cells to be of relatively normal morphology. Labelled nVIII and nVII fibers were observed to enter the brain at ectopic sites. These axons coursed in tracts indistinguishable from those of the controls. HRP-filled terminals on the M-cell could be visualized in the LM and EM: labelled synapses were confined to the usual nVIII and nVII termination zones. Thus, displaced nVIII and nVII axons enter the brain at ectopic locations, course rostrocaudally in characteristic paths and synapse on the appropriate target regions of the ipsilateral M-cell. Axon growth paths are restricted and may organize connectivity patterns by limiting the access of afferents to particular targets. (Supported by NIH grants 5T32GM7288 and NS-18823)

336.1 BLOOD-BRAIN BARRIER (BBB) CHANGES AFTER SYSTEMIC INJECTION OF ALUMINUM. Y. S. Kim*, M. H. Lee and H. M. Wisniewski* (SPON: G. Wen). New York State Office of Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Aluminum in the brains of experimental animals and in humans with dialysis dementia appears to impair cognitive functions. Its role in the pathogenesis of dementia is not yet understood. Recently it has been shown by Banks and Kastin (Lancet ii:1227-1229, 1983) that aluminum increased the permeability of the BBB of rats to small peptides. The purpose of our experiments was to determine whether the BBB also shows permeability changes to the standard tracer, C^{14}-sucrose, in response to aluminum.

Three groups of adult male Sprague-Dawley rats were given an i.p. injection of 100 mg/kg of aluminum chloride, aluminum lactate (in a volume of 2 ml) or physiological saline. Two hours later, 0.5 ml of saline containing 5 μCi of C^{14}-sucrose was injected into the left femoral vein. Plasma radioactivity was measured until the animals were decapitated 10 min after the tracer injection, at which time brain radioactivity was measured in 5 different brain regions. The cerebrovascular permeability and surface area (PA) were calculated by dual compartment model (plasma-brain) of Rapoport et al. (Brain Res., 150: 653-657, 1978). As can be seen in the table below, the PA for C^{14}-sucrose were significantly elevated in all brain regions of the aluminum animals compared to control animals. These findings indicate that acute exposure to a high dose of aluminum alters the permeability of BBB in the rat. Whether in chronic aluminum encephalopathy the BBB is also altered and contributes to the cognitive defect is currently under investigation.

Brain region	PA (sec^{-1} X 10^6) Mean ± SEM		
	Isotonic saline (n=5)	Aluminum chloride (n=6)	Aluminum lactate (n=6)
Cortex	25.4 ± 1.3	71.9 ± 6.0*	73.5 ± 4.6*
Diencephalon	31.7 ± 3.1	62.9 ± 4.5*	75.3 ± 6.4*
Mesencephalon	38.9 ± 4.4	70.4 ± 6.0*	76.7 ± 7.5*
Cerebellum	25.2 ± 3.4	72.6 ± 6.2*	71.7 ± 5.6*
Medulla	37.8 ± 3.7	73.5 ± 6.6*	81.1 ± 7.1*

* P<.01 by Newman-Keul's multiple comparison tests.

336.2 CEREBRAL EDEMA REDUCED BY GM1 GANGLIOSIDE TREATMENT. S.E. Karpiak and S.P. Mahadik. Div. of Neuroscience, NYS Psychiatric Inst. & the Depts. Psychiatry & Biochemistry, Coll. of Phys. & Surg., Columbia U., New York, N.Y. 10032.

Several studies have demonstrated that GM1 ganglioside administration facilitates CNS recovery after injury. One hypothesis is that GM1 facilitates neuronal regeneration in response to injury. Facilitated nerve regeneration has been established morphologically in the PNS [1] but not in the CNS. Our studies suggest alternative hypotheses. We have shown that GM1 facilitates functional recovery after an entorhinal lesion in rat [2]. We also found that at 24hrs post-lesion in rats treated with GM1 mortality rate and the behavioral deficit were reduced. These effects, seen within the time span of 24hrs, could not be explained by increased neuronal sprouting. We postutlated that the facilitated recovery might result from decreased tissue damage at the time of injury. If the damage (cell loss, fiber degeneration) was limited (reduced) by GM1, then the behavioral deficit would be reduced and the longterm recovery would be greater than in controls'. Does GM1 ganglioside reduce the extent of CNS damage occurring after brain trauma?

To examine this we assessed whether GM1 affects levels of edema after trauma. Rats were pretreated for 2 days with GM1 (20mg/kg/i.m.). On day 3 rats (ether anesthesia) were lesioned by inserting a steel rod (3.5mm diam) into one cerebral hemisphere (bregma=0:AP-4mm;L4mm;H5mm) and were given a final injection of GM1. On day 4 levels of edema were measured in 1) the lesioned hemisphere; 2) a tissue punch which circumscribed the lesion area and 3) in hemispheric tissue which excluded the lesion area (punch). Tissue wet weight and dry weight was used to determine the levels of edema (%water). With GM1 treatment, there was a reduction in edema as seen either in the entire injured hemisphere (23%: $p<0.05$) or in locus of injury (33%: $p<0.01$). No effect was seen outside the injured area. Since exogenous gangliosides can spontaneously "insert" into membranes, we postulate that GM1 may be altering membrane processes (lipid hydrolysis; phospholipase activation; levels & membrane action of arachidonic acid; ionic permeation) associated with edema. Studies of effects on these membrane processes may reveal which membrane events are the common mechanisms underlying the multiple effects of ganglioside adiministration.

1. Gorio et al., Neurosci. 8:417(1983).
2. Karpiak, Exp. Neurol. 81:330(1983).

336.3 **NON-EQUILIBRIUM KINETICS OF IODIDE, THYROTROPIN-RELEASING HORMONE (TRH), AND ALBUMIN UPTAKE INTO MOUSE SPINAL CORD FOLLOWING INTRAPERITONEAL INJECTION.** B.R. Brooks, E. Preister*, and D. Sandmire*. Neurology Dept., University of Wisconsin Medical School and W.S. Middleton VA Hospital, Madison, WI 53792.

Drug delivery into the brain has been well studied in an attempt to define the blood-brain barrier, but the spinal cord is a relative "terra incognita" with respect to a number of drugs in general and Thyrotropin-Releasing Hormone (TRH) in particular. We studied the kinetics of uptake following intraperitoneal injection of NaI^{125}, I^{125}-TRH, and I^{125}-bovine serum albumin (BSA) into the peripheral blood compartment, brain, cervical and lumbar spinal cord of NIH:N male mice at fixed intervals up to 60 minutes. First order rates of entry into the peripheral blood compartment at one minute were 13.2% (I), 0.4% (TRH), 0.8% (BSA) of the administered dose. Peak blood levels at 10 minutes were 61.4% (I), 3.9% (TRH), and 28.7% (BSA).

Tissue Uptake at 1 Minute
% Administered Dose/Mg Wet Weight Tissue (x 10^{-5})
Mean (Std. Dev.)

	Spinal Cord		Brain
	Lumbar	**Cervical**	
BSA	**1200** (1100)	**80** (40)	**8** (2)
TRH	**470** (350)	**30** (10)	**10** (1)
I	**2040** (90)	**240** (90)	**40** (10)

Uptake into the brain of the vascular marker (BSA) was equivalent to the uptake of TRH but one fifth that of I. The vascular marker (BSA) uptake was 10 fold higher in the cervical spinal cord and 100 fold higher in the lumbar spinal cord than in the brain. I^{125} uptake into the cervical spinal cord was 3 times that of the vascular marker, and into the lumbar spinal cord was nearly 2 times that of the vascular marker. TRH uptake, however, was decreased by 61% and 62% in the cervical and lumbar spinal cord compared to that of the vascular marker. This decrease was proportional to the degradation rate of TRH (77.4 ± 10.7%/min) following intravenous administration. However, seven times more TRH is present in the lumbar spinal cord following intraperitoneal injection than intravenous injection and correlated with specific TRH related changes in cyclic nucleotides which we have demonstrated in the lumbar spinal cord of mice. (Supported by grants from ALSSOA and MDA.)

336.4 KINETICS OF NEUTRAL AMINO ACID TRANSPORT ACROSS BLOOD-BRAIN BARRIER IN AWAKE RATS L.Miller-, L. Braun-, W. Pardridge and W Oldendorf (SPON:A.Yuwiler). Research Service, Brentwood Hospital, Veterans Administration and Depts of Neurology and Medicine UCLA School of Medicine, Los Angeles, CA. 90073.

Penetration of the blood-brain barrier (BBB) by amino acids (AA) has been shown to be mediated by carrier systems localized on capillary endothelium. These carrier systems were subsequently characterized by their ability to transport a particular class of amino acids, ie neutral, basic or acidic, respectively. Because amino acid availability to the brain depends on transport from blood via membrane bound carriers it is of interest to determine the kinetic characteristics of the transport process. In the past the kinetic constants have been determined using pentobarbital-anesthesized animals. However, it has already been shown that anesthesia will alter not only blood flow but also the rate of glucose transport and utilization. Therefore the present investigation was undertaken to estimate kinetic constants using awake and lightly restrained animals. Our experimental procedure involved anesthetizing the animals with ketamine (150mg/kg,i.m.), exposing the external carotid and inserting a 12cm length of PE-10 tubing filled with heparin. The cannula was externalized through the back of the neck and animals were allowed to recover overnight in separate chambers. On the following day the animals were injected through the cannula with a 200ul bolus of HEPES-buffered Ringers solution,pH 7.5, containing (C^{14})AA and (H^3) water reference. The animals were sacrificed within 5 sec. With each AA 8-9 different concentrations were used from 5uM up to 4mM. Our results for the cortex were:

	Try	Tyr	Phe	Leu	Met	Ile	His	Arg
K_M (uM)	50	64	78	85	101	128	140	67
V_{max} (nmol/min/gr)	23	17	27	26	25	33	28	11
K_D x 10^{-3} (ml/min/gr)	36	42	20	48	36	36	28	23

These constants were obtained from computer analyses of the data points (each point is the average of at least three separate observations) by non-linear regression. For most of the AA's examined the K_M values are significantly lower than published values obtained using animals under pentobarbital anesthesia. Also the low K_M values reported here compared to previous values examining the entire forebrain suggest a greater degree of sensitivity of the brain to transport competitive effects in the physiological range. Present results will be compared with values obtained from three other brain regions: hippocampus, caudate and thalamus/hypothalamus.

336.5 MAJOR BLOOD-BRAIN BARRIER PHOSPHO-PROTEINS. W. Cefalu*, J. Yang* and W.M. Pardridge (SPON: S.G. Diamond). Dept. of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

The regulation of blood-brain barrier permeability is likely regulated in part by the phosphorylation of plasma membrane and cytosol proteins in brain microvessels. In our initial attempts to characterize major BBB phospho-proteins, we isolated microvessels from rat and bovine brain using a mechanical homogenization technique. The microvessels were ruptured by hypotonic lysis and the cytosol released with this procedure was collected and concentrated. The plasma membranes were separated from the basement membranes by sonication. The plasma membrane fraction was enriched in a gamma glutamyl transpeptidase, alkaline phosphatase, insulin receptor binding and produced rabbit antisera that bound to lateral borders of isolated endothelial cells. SDS-polyacrylamide gel electrophoresis (PAGE) of the rat or bovine BBB plasma membrane fraction showed that both species demonstrated major bands at 46K, 68K and >200K molecular weight. The plasma membranes were reacted with γ-^{32}P-ATP and incubated at 30°C for 5 min. The phospho-proteins formed with this procedure were separated by SDS-PAGE and were analyzed by autoradiography. The major phospho-proteins in the BBB plasma membrane fraction of either rat or bovine brain were doublets at 55K and 58K and a major phospho-protein of >200K. Minor phospho-proteins were also noted with molecular weights of 19K, 46K, 72K, and >100K. SDS-PAGE analysis of the rat or bovine brain microvessel cytosol revealed numerous bands with substantial qualitative similarities between the two species. Major bands were noted of molecular weight of 48K and 68K. The 68K protein was by far the most abundant protein in the BBB cytosol of either species. The cytosol proteins were labeled with γ-^{32}P-ATP at 30° for 5 min and were analyzed by SDS-PAGE and autoradiography. At least 15 different phospho-proteins were detected in the cytosol of either species and the major phospho-protein comigrated with the major protein seen on the croomasie blue gels at molecular weight = 48K. The principal cytosol protein of molecular weight of 68K seen on the croomasie blue gel was not a phospho-protein. In conclusion, these studies demonstrate the presence of multiple phospho-proteins in both the plasma membrane and the cytosol fraction of the blood-brain barrier. The function of these proteins, in particular, the 55K and 58K doublet in the plasma membrane, and the 46K protein in the cytosol remain to be elucidated. These studies also demonstrate that the most abundant protein in the BBB cytosol is a 68K non-phospho-protein.

336.6 RAPID SEQUESTRATION AND DEGRADATION OF SOMATOSTATIN ANALOGUES BY ISOLATED BRAIN CAPILLARIES. J. Eisenberg*, W.M. Pardridge and T. Yamada* (SPON: H. Weiner). Depts. of Medicine, UCLA School of Medicine, Los Angeles, CA 90024, and Univ. of Michigan School of Medicine, Ann Arbor, MI 48109.

The mechanism by which neuropeptides are inactivated after release into the interstitial space is at present unclear. The possibility that brain microvessels may play a role in the rapid inactivation of neuropeptides was investigated in the present studies. Capillaries were isolated from bovine brain with a mechanical homogenization technique. ^{125}I-tyr-1-somatostatin (SRIF) was rapidly metabolized at 23°C by isolated brain microvessels by aminopeptidase with a half-time of less than 2 minutes in the presence of 1.1 mg capillary protein. The aminopeptidase degradation of tyr-1-SRIF was saturable with a K_m of 76 μM and a V_{max} of 7.4 nmol/min/mg protein. Since rapid metabolism of tyr-1-SRIF would prevent assessment of endothelial uptake mechanisms, another analogue of SRIF was used: ^{125}I-tyr-11-SRIF. TYR-11-SRIF was rapidly sequestered by microvessels. The sequestration process reached equilibrium by less than 5 seconds at 0°C or 37°C. The sequestration process was non-saturable up to 1 μg/ml SRIF and was not inhibited by other basic peptides. The amount of SRIF sequestered by the microvessels decreased with time at 37°C. The decreased binding of SRIF to the microvessels was associated with enhanced formation of iodo-tyrosine as judged by HPLC analysis of the medium and cell extract at various times of incubation. The rapid sequestration of SRIF by the microvessels obtained with a mechanical homogenization technique was also observed in bovine brain microvessels obtained with a dispase enzymatic homogenization technique which yields microvessels that are trypan blue negative. The microvessel cytoskeleton remaining after 1% Triton X-100 extraction also rapidly bound ^{125}I-tyr-11-SRIF and this binding was resistant to alkaline pH. Finally, the rapid sequestration of SRIF was specific since microvessels bound very little ^{125}I-bradykinin, another basic peptide, and bound essentially no ^{125}I-CCK8, an acidic neuropeptide. In conclusion, these studies demonstrate that some neuropeptides such as SRIF are rapidly sequestered by microvessels and this sequestration process would appear to facilitate enzymatic degradation of the peptide by brain capillary associated proteases. The Triton extraction experiment suggests the binding process represents a peptide interaction with the endothelial cytoskeleton.

336.7 MULTIPLE USES OF THE CAROTID INJECTION TECHNIQUE WITH N-ISOPROPYL-p-(^{125}I)IODO AMPHETAMINE (IMP) AS AN INTERNAL REFERENCE. G. Fierer* and W.M. Pardridge (SPON: D.E. Kuhl) Dept. of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

The ideal reference for the carotid artery injection technique is a substance that is 100% cleared by brain from blood and is also actively sequestered by brain such that essentially none of the compound effluxes back to blood during short experimental time periods. Moreover, the reference should be ^{125}I-labeled so that the blood-to-brain transport of ^{3}H and ^{14}C compounds can be studied simultaneously. A candidate for the ideal ^{125}I reference is IMP. In the present studies, quench correction curves were first computed for triple isotope (^{3}H, ^{14}C, ^{125}I) liquid scintillation counting. Transport studies were performed in both ketamine anesthetized adult rats and in external carotid artery catheterized conscious rats. Three isotopes, ^{3}H-water, ^{14}C-butanol, and ^{125}I-IMP, were mixed in a ratio of 50/10/2 μCi/ml in Ringer-Hepes buffer and were rapidly injected in a 0.2 ml bolus in the carotid artery. Brain uptake indices (BUIs) were computed from the ^{3}H/^{125}I and the ^{14}C/^{125}I ratios in brain divided by the same ratio in the injection solution at 5, 15, 30, and 60 seconds after injection. The ln of the BUI for water or butanol increased linearly with time. The blood-to-brain and the brain-to-blood transport of water and butanol was assessed from the intercept (Emax) and slope (K), respectively:

condition	Emax(HOH)	Emax(but)	KHOH(min^{-1})	Kbut(min^{-1})
ketamine	0.73	0.82	0.90	0.99
conscious	0.55	0.87	0.99	1.78

The ^{125}I radioactivity in brain was constant over the 60 second time period for both the anesthetized and the conscious animal. This constancy of the ^{125}I radioactivity allows for computation of blood-brain barrier transport kinetics in either direction across the barrier simply from the BUI ratios. These studies demonstrate that ^{125}I-IMP may be an ideal reference for use in the carotid injection technique. These studies also show that ^{14}C-butanol is not freely transported through the blood-brain barrier in either of the two conditions studied. Therefore, there is a need for either a ^{3}H or ^{14}C compound that is 100% cleared by brain but is not sequestered by brain binding systems. With such a compound, the simultaneous extraction and blood flow can be determined with the carotid artery injection technique in individual rats at a single time point.

336.8 IONIC HOMEOSTASIS OF THE CHOROID PLEXUS-CSF SYSTEM IN GANGLIONECTOMIZED OR ADRENALECTOMIZED RATS STRESSED WITH ACIDOSIS. C.E. Johanson and R.E. Harbut*. Dept. of Pharmacology, Univ. of Utah Sch. Med., Salt Lake City, UT 84132.

Acute systemic acidosis causes substantial changes in lateral ventricle choroid plexus (LVCP) epithelial cell content of K (increased) and Na (decreased), and a slight reduction in CSF [K]. Such an effect has been linked to an elevated titer of extracellular catecholamines consequent to induction of blood acidosis. To test this hypothesis, we induced acute metabolic acidosis (4.7 mmol/kg NH_4Cl, IP, 30 min.) in ketaminized (100 mg/kg) adult, Sprague-Dawley rats depleted of catecholamines either locally (by superior cervical ganglionectomy) or systemically (by adrenalectomy); then, the ionic composition of CP tissues and cisternal CSF was analyzed to ascertain deductively the role of catecholamines in the blood-CSF barrier response to systemic acid-base imbalance.

Superior Cervical Ganglionectomy (SCGx).

Both ganglia were surgically exposed, but only one ganglion was removed; unilateral ptosis confirmed the success of the aim to ablate functionally the innervation to one LVCP, but not to the contralateral CP. SCGx for 6 days did not alter CSF [K] (3.08 mM) or [Na] (157 mM); nor did it alter the [K] or [Na] in the CP of either the denervated or intact side. Injection of NH_4Cl to induce metabolic acidosis caused a substantial elevation in [K]/[Na] in the CP tissues of both lateral ventricles. Thus, acidosis-induced changes in tissue ion content observed in SCGx-LVCP was the same as those in sham SCGx-LVCP.

Adrenalectomy (ADRx).

Following bilateral ADRx, the adult rats had free access to food and water. One day after ADRx, the animals were injected with either NaCl (control) or NH_4Cl for 30 min. Bilateral adrenalectomy did not block the effect of NH_4Cl to increase CP [K]/[Na].

Thus, the results from both the "local" and "systemic" adrenergic ablation experiments indicate that catecholamines probably do not play a major role in the unique ability of the CP to retain K in the face of acute systemic metabolic acidosis. Supported by NIH Grant NS 13988.

336.9 EFFECT OF TREATMENTS WHICH ALTER Na-22 UPTAKE INTO CSF ON Na-22 UPTAKE INTO BRAIN REGIONS PROXIMATE OR DISTANT TO THE VENTRICLES. V.A. Murphy* and C.E. Johanson (SPON: A.B. Butler). Dept. of Pharmacology, Univ. of Utah, Salt Lake City, UT 84132.

Smith, Tal. and Rapoport (Soc. Neurosci., 1983) have shown that ion uptake from plasma into brain regions distant from the ventricles is mostly direct from capillary plasma rather than indirect from CSF; regions closer to the ventricles had greater contribution of ion uptake from CSF. To determine if uptake into certain regions of the brain is influenced predominately by changes in uptake of Na-22 by CSF or by cerebral capillaries, treatments which alter CSF uptake of Na-22 were administered to nephrectomized male adult rats. Then, isotope uptake into cerebral cortex (distant from ventricles) and medulla (near the fourth ventricle) as well as CSF was measured.

Na-22 0.05 mCi/kg was administered IP either 0, 12. 24, 36. 42. or 48 min. after treatment. HCl or NH_4Cl, 4.7 mmol/kg; acetazolamide, 25 mg/kg; or amiloride, 100 mg/kg injected IP were treatments used. Animals were sacrificed 1 hr after drug injection. Volume of distribution (Vd) was dpm/g tissue (or CSF) over dpm/g plasma H_2O x 0.95. Na-22 uptake was the product of slope and Vd at time 0 obtained from a plot on a log-linear scale of the difference of steady-state Vd of Na-22 and Na-22 Vd, against time. Since Na-22 did not reach steady state in 1 hr, stable Na Vd was used for this value (mmol Na instead of dpm in Vd equation).

Acetazolamide. amiloride. and HCl reduced early uptake of Na-22 into CSF by about 30% while NH_4Cl reduced uptake by 12%. Cerebral cortex uptake of Na-22 was unaltered by acetazolamide. lowered 20% with amiloride or NH_4Cl, and decreased 30% by HCl. Uptake into medulla was depressed about 20% by all treatments except HCl which reduced uptake by 36%.

Acetazolamide does not alter cerebral capillary permeability; therefore uptake into distant regions minimally affected by reduction in CSF uptake is unaltered while proximate brain region uptake is reduced due to a significant contribution of Na from CSF. Amiloride which slows Na transport by inhibition of Na-H exchange reduced distant uptake as well as proximate which suggests some Na transport across cerebral capillaries is by Na-H exchange. Acidosis (NH_4Cl or HCl) resulted in greater effects on brain uptake than expected from changes in CSF uptake which implies that acidosis is changing cerebral capillary permeability or blood flow. Supported by NIH Grant NS 13988.

336.10 EVALUATION OF CEREBRAL ENDOTHELIAL VESICLES BY SERIAL SECTIONING. B.L.Coomber and P.A.Stewart. Dept. of Anatomy, University of Toronto, Toronto, Ontario, CANADA. M5S 1A8.

Bulk trasnport of blood-bourne substances across endothelial cells is thought to occur via shuttleing of isolated pinocytotic vesicles, but the role of such vesicles in bulk transport has recently been questioned. Serial sections of microvessel endothelium from somatic tissues has shown that most vesicles are connected to tubules or other vesicles, forming chains within the cytoplasm. Our study attempts to elucidate the three dimensional arrangement of pinocytotic vesicles within the endothelium of cerebral microvessels.

Serial sections of mouse cerebral cortex were collected on Formvar coated slot grids. Considerable care was taken to obtain very thin sections, and section thickness was estimated from interference colours to be less than 30 nm (very dark grey). Grids were examined with TEM and suitable areas of microvessels were photographed. Acetate tracings from subsequent micrographs were used to follow vesicular structures through adjacent sections.

Although almost all vesicles examined appeared as isolated structures in the endothelial cytoplasm when a single section was examined, the majority of them were actually connected to other vesicles, to golgi, or to ER-like tubule complexes. After examing adjacent sections, we estimate that only one third of all vesicles are "free" in the cytoplasm. Furthermore, our sections may have been too thick to detect strictures between some fused vesicles, so the actual number of isolated vesicles may be even lower than this. Complex clusters of vesicles, as described in endothelium from other tissues, were also not seen in our study. The results suggest that the number of isolated pinocytotic vesicles observed in cerebral microvasculature endothelium may be artifactually high if only single sections are examined.

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336.11 Morphometry of Mitochondria and Endothelial Cells in Brain Capillaries Following Alterations in Serum $[K^+]$ Levels (SPON: H.E. Hirsch). W.H. Oldendorf, L.A. Paul , J. Eifert . Brentwood VA Hosp. and Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90073.

The blood-brain barrier (BBB) is the result of several properties of the capillary endothelial cell. How do these cells maintain a stable brain extracellular (EC) milieu in the face of dramatic fluctuations in levels of circulating ions? Brain EC $[K^+]$ remains constant during wide variations in the plasma-brain $[K^+]$ gradient. Since this gradient is across the brain capillary endothelial cell, high serum $[K^+]$ is hypothesized to increase pumping by the BBB to maintain normal EC levels. This changing metabolic work could be reflected in endothelial cell morphology, particularly in mitochondria which have been shown to be 3-4 times more numerous in brain capillary endothelial cells than in other capillary endothelial cells.

We measured several morphologic parameters of brain capillary endothelial cells in white male New Zealand rabbits following dietary manipulation of their serum $[K^+]$ levels for 3 weeks. Four groups, based on serum $[K^+]$ levels read approximately twice weekly, were formed: Deficient, Low, Normal, and High. Following the final day's weight and blood samples, rabbits were anesthetized and perfused for transmission electron microscopy. This abstract reports on data from sensory motor cortex.

Capillaries were identified and photographed according to predetermined criteria and planimetric measurements were made with a rolling disk planimeter. Variables included number and area of mitochondria, areas of capillary lumen and capillary diameter, area of endothelial cell, and proportion of endothelial cell taken up by mitochondria. All data were analyzed using a one-way Analysis of Variance program (BMDP7D).

No differences appeared among Deficient, Low, Normal, or High groups in Diameter of Endothelial Cell, Diameter of Lumen, Number of Mitochondria per Photograph, or Proportion of Endothelial Cell Area Occupied by Mitochondria. The groups did differ, however, in Area of Endothelial Cell ($p<0.01$), with the size of the cell consistently decreasing as serum potassium increased.

Lack of anticipated differences in mitochondrial number or proportional area indicates either that pumping K^+ occupies a minor fraction of cell energy, or that our measurements are not sufficiently sensitive indicators of metabolic workload in this case. It is also possible that plasticity in brain capillaries is expressed by alterations in cell size, rather than in mitochondrial parameters. One effect of smaller endothelial cells and constant mitochondrial size could be that concentrations of ATP within the cell are increased.

336.12 PERMEABILITY AND MORPHOLOGICAL CHANGES IN THE ENDONEURIAL VASCULATURE OF THE FROG SCIATIC NERVE DURING WALLERIAN DEGENERATION. C.H.Latker*, A. Weerasuriya*, D.M. Jenkins* and S.I. Rapoport. (Spon: N.L. Shinowara) Lab. of Neurosciences, National Institutes on Aging, NIH, Bethesda, Maryland 20205.

During Wallerian degeneration an increase in permeability of the blood-nerve barrier (BNB) to Evans Blue albumin occurs at the site of the lesion. We investigated the permeability and morphology of the endoneurial capillaries of the transected and contralateral sciatic nerve of adult Rana pipiens. The proximal stump at the site of transection was tied to exclude regenerating fibers from penetrating the distal segment. The permeabilities of ^{14}C sucrose and horseradish peroxidase (HRP) were assessed at 3 days to 6 weeks after transection. In the transected nerve capillary permeability to ^{14}C sucrose started to increase at 1 week and showed a 4 to 6 fold increase by 3 weeks. In the contralateral nerve, an increase of at most 50% was seen during the corresponding time periods. Additional animals were injected with HRP, the nerves were removed and processed by standard histochemical methods. In the degenerating segment of nerves lesioned, 1 and 3 weeks earlier, HRP reaction product (HRP-RP) filled the endoneurial space. In the corresponding contralateral nerve, HRP-RP was detected only in the perivascular area. Tracer was detected on both the luminal and abluminal surfaces of the endothelial cells, filling the surface caveolae and vesicular profiles within the cells, and the interendothelial space. In nerves from control animals HRP-RP was confined to the lumen of the endoneurial blood vessels. Because the above changes began to appear only about a week after the transection it is suggested that they were related to the degenerative changes in the nerve and not to the trauma associated with the transection. These finding suggest that within the endoneurial space vascular integrity is dependent on intact neural components.

336.13 BOVINE BRAIN CAPILLARY ENDOTHELIAL CELLS IN PRIMARY CULTURE: EXPRESSION OF MEMBRANE POLARITY AND γ-GLUTAMYL TRANSPEPTIDASE ACTIVITY. J. Yang* and W.M. Pardridge (SPON: D.S. Maxwell). Dept. of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

Brain capillary endothelia express unique biochemical and morphologic characteristics similar to polarized transporting epithelial systems. Two characteristics of transporting polarized barrier systems are the expression of γ-glutamyl transpeptidase (γ-GTP) and the asymmetric distribution of surface antigens to apical and basolateral membranes. These two properties were assessed in bovine capillary endothelial cells grown in primary tissue culture using the method of Bowman et al (Ann. Neurol. 13, 396, 1983). In this method, microvessels were obtained from bovine brain homogenized with a 3 hour treatment of dispase/collagenase, followed by dextran centrifugation. The microvessels were converted into an enriched endothelial cell population by an overnight collagenase (0.1%, 37°C) digestion, followed by percoll density gradient centrifugation. The cells migrating as a single band on the percoll gradient were cytospun onto glass slides and were analyzed for γ-GTP activity using a histochemical technique that utilizes γ-glutamyl-4-methoxy-2-napthylamide as substrate. This cell population was shown to be more than 90% positive for γ-GTP activity. The endothelial cells were plated onto collagen-coated petri dishes and were grown in primary tissue culture for 10-14 days. The level of γ-GTP activity in the cultured endothelial cells was comparable to the activity seen in rat C6 glioma cell line cultures which are known to be positive for the enzyme. Asymmetric localization of surface antigens to the lateral cell membrane of the brain endothelial cells in culture was shown using a rabbit antisera prepared against bovine brain capillary plasma membranes in an avidin/biotin/peroxidase technique. At a primary antisera dilution of 1:100 the lateral membranes and cell-to-cell contact areas were selectively visualized with the antisera, whereas preimmune rabbit sera showed no asymmetric distribution. These studies show that two characteristics of the blood-brain barrier in vivo, γ-GTP activity and asymmetric surface antigen distribution, are maintained in bovine brain endothelial cells in primary culture. This model system should prove useful in future studies of the genetic regulation of the biochemical characteristics of the blood-brain barrier phenomena in primary tissue culture.

336.14 PERINEURIUM OF FROG PERIPHERAL NERVE:A ROLE IN REGULATING ENDONEURIAL CALCIUM? K.C. Wadhwani, H. Levitan and S.I. Rapoport, Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20814

Since many functions of peripheral nerves, such as axonal transport and excitability, are sensitive to calcium the concentration of calcium in the endoneurial space surrounding the nerve fasicles may be controlled by the blood-nerve barrier. To determine if the nerve sheath (perineurium) is actively involved in regulating calcium in the endoneurial space we have examined the flux of ^{45}Ca across a perfused perineurial cylinder isolated from a segment of frog sciatic nerve, and into a nerve segment bathed in situ in Ringers containing radiotracer. The flux of ^{45}Ca was compared to that of ^{3}H-sucrose measured simultaneously, since sucrose is thought to passively permeate the tissue. In all cases the physiological solution contained 5 mM glucose, was aerated with 95% O_2/5% CO_2, and buffered with HEPES to pH 7.3. The mean permeability for ^{45}Ca influx across the isolated perineurium was $(9.7\pm0.6)\times10^{-7}$ cm/s (n=13) compared to a permeability for ^{3}H-sucrose influx of $(7.0\pm0.4)\times10^{-7}$ cm/s. The mean permeabilty for ^{45}Ca efflux was $(26.6\pm6.1)\times10^{-7}$ cm/s compared to $(17.4\pm4.6)\times10^{-7}$ cm/s for sucrose. Although the permeabilty for calcium efflux exceeded that for influx the mean ratio of the permeability for ^{45}Ca efflux to influx in individual tissues (2.7 ± 0.6) was not signficantly different from that of sucrose (2.4 ± 0.6). Varying the calcium concentration in the Ringers from 0 to 10mM, perfusing with Na-free Ringers, and adding ouabain, had no effect on the flux of calcium or sucrose in either direction through the tissue. We concluded that asymmetric flux of tracer through the isolated cylinder was due to the slight (~2mm Hg) hydrostatic perfusion pressure rather active efflux of calcium. To determine the extent to which the procedure of isolating the perineurial cylinder from the nerve fasicles affected the permeability characteristics of the sheath we examined the uptake of ^{45}Ca and ^{3}H-sucrose into the endoneurial space after incubating a segment of nerve for 30 min in situ in a pool of Ringers containing these radiotracers. Although the permeability of the perineurium to influx of calcium and sucrose, $(3.0\pm0.4)\times10^{-7}$ and $1.5\pm0.2)\times10^{-7}$ cm/s, respectively (n=18), were about a third that found in the isolated tissue, the ratio of the permeability of calcium to sucrose (2.0 ± 0.1) was not significantly different. The results suggest that the perineurium is passively permeable to calcium as well as sucrose.

336.15 SPINAL CORD CONTUSION IN THE RAT: ALTERATIONS IN VASCULAR PERMEABILITY TO HORSERADISH PEROXIDASE (HRP). L.J. Noble and J.R. Wrathall. Department of Anatomy, Georgetown University, School of Medicine, Washington, D.C. 20007.

The integrity of the blood-spinal cord barrier to HRP has been examined 3 hours after a concussive injury produced by a 10 gm weight which was dropped 2.5, 5.0, or 17.5 cm onto the exposed dura. HRP was dissolved in 0.9% saline and injected (150mg/kg) into the jugular vein 10 minutes prior to sacrifice of the rat. Diphenhydramine hydrochloride (Benedryl), a histamine antagonist, was given intraperitoneally 15 minutes prior to the tracer injection.

In addition to the physical disruption of blood vessels as a result of the mechanical trauma, endothelial cells may alter their permeability to macromolecules in response to the trauma. Previous studies, using a transection model in the rat, have demonstrated that endothelial permeability to HRP is a transient feature (lasting from 3 to 12 hours) of the intrinsic vasculature and occurs at sites which are not directly traumatized by the transection. Our preliminary observations at the light microscopic level suggest that a similar vascular response may occur after a concussive injury. Certain vessels exhibited a halo of reaction product which extended into the adjacent extracellular spaces. Although this distribution of HRP was often associated with vessels at the epicenter, it also occurred around vessels away from the impact site. The limited resolution precluded further refinement of the tracer localization. In general the gray matter appeared to contain more reaction product than the white matter. Three hours after a concussive injury, there was evidence of reaction product in the interstitium of the gray matter up to at least 2 cm from the impact site. It was not unusual to find neuronal uptake of HRP, especially in neurons adjacent to the central canal and in ventral horn motoneurons. The region of the dorsal columns associated with the corticospinal tracts was particularly labelled with both scattered erythrocytes and reaction product. HRP was also present in the central canal after each injury and was detected up to at least 2 cm from the impact site after the 5.0 and 17.5 cm weight drop injuries. In certain cases, the ependyma also appeared labelled with HRP. Since ependymal cells do not provide any significant barrier to HRP, the central canal may act as a conduit for the transport of exogenous proteins into the surrounding gray matter. Ultrastructural studies are underway to further define these observations.

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336.16 THE MECHANISM BEHIND THE BLOOD BRAIN BARRIER OF THE COCKROACH NERVE CORD. D.B. Henken and S.R. Shaw, Dept. of Psychology, Dalhousie University, Halifax, N.S., Canada.

Insects share with vertebrates the common property of having a CNS protected by a blood brain barrier. They also share a common mechanism according to a hypothesis of long standing; this supposes that at the boundary between blood and CNS, the extracellular spaces are sealed off by tight junctions between special cells (perineurial cells in insects; review: Lane (1981), Int. Rev. Cytol. 73, 243). Studies on the blood retinal barrier of insects failed to support this view. We therefore re-examined one of the preparations from which evidence for the tight junction hypothesis was originally drawn, the ventral nerve cord of the roach Periplaneta. After exposing the cord in situ to a saline containing ionic lanthanum, we confirmed the earlier findings that this extracellular tracer is arrested just below the surface of the cord, indicating that the diffusion barrier there remained intact. The superficial monolayer formed from numerous perineurial cells was readily identified, but contained no obvious intercellular junctions, and was everywhere circumvented by tracer, contrary to expectations. Perineurial cells do not contribute to the barrier system. Underneath this layer lies another monolayer of previously unrecognized sheath cells. These are very thin but extend circumferentially for great distances; six of them are sufficient to encircle the cord completely, leaving only six clefts for diffusional access. They make interdigitating contacts with each other and form the outer edge of the barrier, but serial EM sections of these sites failed to reveal any tight junctions, only extensive pleated septate junctions. Re-evaluation of the illustrations in the large literature on the barrier also failed to disclose any convincing examples of tight junctions between superficial cells in the cord.

We propose that at this outermost level, a barrier is created solely by a dimensional effect: extreme constriction and lengthening of the overall extracellular pathway, as this runs between sheath cells within septate junctions. With the dimensions measured, the half-time to fill the center of the cord with La^{3+} should be >7 days, calculated from an approximation to the diffusion equation; for a smaller, biologically important ion like K^+, $t_{\frac{1}{2}}$ > 3 days. These values are easily long enough to explain the experimental findings to date. This result eliminates the need to postulate special occluding junctions to account for the barrier in insects. Supported by NSERC A9593, Canada.

336.17 EXAMINING BLOOD-BRAIN BARRIER PERMEABILITY TO HORSERADISH PERODIXASE AND TO α-AMINOISOBUYTRIC ACID FOLLOWING ACUTE HYPERTENSION: A COMPARATIVE, QUANTITATIVE, AND MORPHOLOGICAL STUDY. M.D. Ellison*, J.T. Povlishock, and R. Hayes (SPON: J. Johnson). Dept. of Anat. and Div. of Neurol. Surg., Med. Coll. of Va., Va. Commonwealth Univ., Richmond,VA 23298.

The blood-brain barrier (BBB) selectively restricts the blood-to-brain passage of many solutes owing to unique properties of endothelial cell membranes. Normal BBB function is altered under various conditions, allowing the transfer into brain parenchyma of substances normally excluded. To date, tracers most commonly used to study cerebrovascular permeability changes have included horseradish peroxidase (HRP) and vital dyes bound to serum albumin. To supplement information provided by such studies, a new BBB technique has been developed employing as a tracer a synthetic small neutral amino acid, α-aminoisobutyric acid (AIB), MW 104. When radiolabeled with ^{14}C, AIB can be used as a BBB tracer for autoradiography. The nature of the technique allows the calculation of a regional, unidirectional blood-to-brain transfer constant, Ki, for AIB, allowing a quantitative expression of vascular permeability to the tracer in any brain region.

Employing acute hypertension as a model of BBB disruption in rats, changes in permeability to both HRP and AIB were examined in each animal. Extensive analyses of regional transfer constants for AIB were performed. The topographical distibution of tracer extravasation sites and their correlation were studied for each probe. The data revealed dramatic focal permeability increases to AIB in certain brain regions ($Ki \geq 30$ ml/g/sec x E^5) which, in adjacent sections processed for HRP visualization, also showed HRP extravasation. However, other brain regions showed more diffusely distributed and subtly elevated AIB passage ($2 < Ki \leq 30$ ml/g/sec x E^5) where no corresponding HRP passage was observed. In additional animals subjected to acute hypertension, comprehensive ultrastructural studies of cerebral microvessels were undertaken to examine the possible morphological correlates of the observed barrier alterations. Interendothelial junctions and endothelial membranes appeared intact; however, numerous protein-filled vesicles were observed in those vessels extravasating protein. The results of this investigation suggest that protein studies alone do not reveal all aspects of altered barrier status and that multiple mechanisms of increased BBB permeability may operate simultaneously in the absence of frank cellular disruption.

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336.18 DURAL MAST CELLS: DISTRIBUTION, MORPHOLOGY AND PATHOPHYSIOLOGY. E.L. Orr* and J. Aschenbrenner* (SPON: D.J. Barker) Depts. of Anat. and Biol. Sci., Texas Coll. Osteop. Med. and North Tex. St. Univ., Fort Worth, TX. 76107.

The presence of mast cells in the meninges of several species has been demonstrated. In particular, the dura mater of the mouse contains large numbers of mast cells, which massively degranulate under certain experimental conditions and thereby release their contents (e.g. histamine) onto or near the brain (Orr, J. Neurochem., in press). Since histamine (and other mast cells products) can affect neuronal excitability as well as the permeability of the blood-brain barrier (BBB), it seems likely that menigeal mast cells may affect brain function under certain physiological or pathological conditions. Thus, to begin evaluating the significance and role of menigeal mast cells in brain function, we are investigating the number, distribution and morphology of mast cells in the dura mater of the mouse, and are beginning studies of their significance after brain injury or insult.

Our initial studies have concentrated on the dura mater overlying the parietal cortex. Whole amounts of dura were prepared, fixed in neutral buffered formalin, and stained with acidified 0.5% toluidine blue. Mast cells were identified by their metachromasia and presence of large numbers of granules. Counts of 6 parietal dural spreads from 3 mice indicated that there were 27.3 ± 1.8 mast cells/570μ^2 of dura ($\bar{X}$±SEM), with most of the mast cells located adjacent to the dural blood vessels. Further, cross-sections of dura were prepared and demonstrated that the mast cells were located in an intermediate position between the periosteal and subdural surfaces of the dura. Furthermore, the local application of a liquid nitrogen-cooled brass rod to the surface of the skull is a commonly used model for investigating the effects of brain injury on (e.g.) breakdown of the BBB (c.f., Baker, et al, J. Neuropath Exp. Neurol 30:668, 1971). Using this paradigm, we have found that dural mast cells are largely degranulated within minutes in the area of freezing, while adjacent mast cells remain intact. We are presently determining the exact time course of degranulation and the change in dural and cerebral histamine levels in relation to breakdown of the BBB induced by cold injury. Light and electron micrographs demonstrating these features will be presented.

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MONOAMINES AND BEHAVIOR: DOPAMINE

337.1 NEURAL SUBSTRATES FOR THE BEHAVIORAL OUTPUT OF THE NUCLEUS ACCUMBENS. N.R. Swerdlow[1]* and G.F. Koob[2]. [1]M.S.T. Program, School of Med. U.C.S.D., La Jolla, CA 92093 and [2]Div. Neurosci. and Endocrin., Scripps Clinic and Research Foundation, La Jolla, CA 92037

The "supersensitive" locomotor response to apomorphine (APO) following 6OHDA-induced denervation of the nucleus accumbens (NAc) is believed to be mediated via NAc efferents onto cells within the substantia innominata (SI), a projection known to contain the transmitters GABA, enkephalin and substance P. It is not known which of these transmitters serves to transmit information responsible for this locomotor response, nor is it known how cells within the SI transmit this information to lower motor circuitry. We further examined the neurochemical and anatomical substrates of the "supersensitive" locomotor response.

In one experiment, animals (n=14) received 6OHDA injections into the NAc. One week later, locomotor responses to 0.1 mg/kg APO were measured on two successive days following sc injection of either saline or 5 mg/kg naloxone. Naloxone pretreatments did not alter the "supersensitive" locomotor response.

In a second experiment, animals (n=18) were divided into two groups that received injections of either 6OHDA or vehicle into the NAc and implanted with cannulae above the SI. One week later, locomotor responses to APO were measured on four successive days following infusion of either 0, 10, 50 or 100 ng of the GABA agonist muscimol into the SI. Muscimol decreased the locomotor response to APO, and at higher doses produced a subsequent prolonged dose-dependent locomotor activation.

In a third experiment, all animals received 6OHDA injections as above. One group (n=23) received injections of ibotenic acid or vehicle into SI terminal regions within the pedunculopontine nucleus (PPN). Two other groups received electrolytic or sham lesions of the medial frontal cortex (MFC, n=14) or dorsomedial nucleus of the thalamus (DMT, n=16). Lesions of the DMT, but not PPN or MFC, significantly attenuated the supersensitive locomotor response.

Our results indicate that inhibition of GABA transmission from the NAc to the SI is a substrate for locomotor activation involved in the "supersensitive" response to APO, and that this effect is translated to lower motor circuitry through SI efferents to or through the DMT. This circuitry may be an important substrate by which mesolimbic activity is translated into behavior.

337.2 INTRA-ACCUMBENS METHYLPHENIDATE INJECTIONS FAIL TO PRODUCE PLACE PREFERENCE CONDITIONING. M.T. Martin-Iverson and H.C. Fibiger. Div. Neurol. Sci., Dept. Psychiat., University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

It was previously reported (Martin-Iverson, M.T. et al., Soc. Neurosci. Abstr., 1983) that the locomotor stimulant effects of systemically administered methylphenidate (MPD) were pharmacologically distinct from its reinforcing properties as assessed by conditioned place preference (CPP). Furthermore, while systemically administered d-amphetamine (AMP) produces CPP which can be attenuated by dopamine (DA) antagonists, MPD-induced CPP is more resistant to such treatments. Evidence from a variety of sources indicate that the reinforcing properties of AMP may be dependent upon an action on DA terminals in the nucleus accumbens. This appears to be true for both CPP (Spyraki, C. et al., Brain Res., 253:185,1982) and intravenous self-administration (Lyness, W.H. et al., Pharmac. Biochem. Behav., 11:553,1979). Intra-accumbens AMP microinjections can produce CPP (Carr, G.D. and White, N.M., Life Sci., 33:2551,1983), while microinjections into the striatum do not, further suggesting that the rewarding effects of AMP are specific to actions on the mesolimbic DA system.

The purpose of the present experiment was to determine if CPP produced with MPD can be differentiated from that produced with AMP on the basis of site of action. Thus, the CPP procedure was used to examine the reinforcing action of intra-accumbens injections of MPD (20 µg) dissolved in 0.5 µl of saline in 9 rats, as compared to 9 rats injected with saline. The procedure of Spyraki et al. (1982) was followed with 3 exceptions: 2 pretests were conducted, relatively neutral cues were used to distinguish the two sides of the test boxes, and the post-conditioning test (Test 1) was followed on a subsequent day by a test preceded with MPD or saline intra-accumbens injections (Test 2).

It was found that rats did not show significant preferences for the side of the box associated with intra-accumbens MPD injections, either on Test 1 or Test 2. However, the number of crossings from 1 side of the box to the other increased significantly after MPD injections on Test 2. Thus, while intra-accumbens MPD injections are effective in producing locomotor stimulation, they fail to produce CPP, at least with the dose and regimen used in the present study. This suggests that the rewarding properties of MPD may depend on a localization at some site other than the nucleus accumbens.

337.3 THE ROLE OF FOREBRAIN DOPAMINE IN BRAIN STIMULATION REWARD. Robert J. Carey. VAMC at Syracuse, Syracuse, NY 13210

Interference with brain dopamine neurotransmission can severely impair brain stimulation reward behavior. The significance of this impairment in reward behavior, however, has been a problematic issue. That is, it has been difficult to decide whether the dopamine dysfunction has attenuated the reward effect of the stimulation or has merely rendered the animal less able to generate the behavior required to obtain reinforcement. To experimentally re-assess this issue, the present studies examines the effect of neuroleptic drugs on brain stimulation reward in animals with unilateral 6-hydroxydopamine lesions of forebrain dopamine neurons. Rats with bilateral medial forebrain bundle electrodes which generated comparable rate-intensity functions for self-stimulation were administered unilateral injections of 6-OHDA (4 µl of a 3 µl/µl sol.) into the substantia nigra and ventral tegmental area. Initially, the effect of the severe unilateral dopamine depletion was manifested by a drastic bilateral decrease in self-stimulation which gradually recovered over a two month period. When equivelent performance was re-established in each hemisphere electrode site the rats were given either 0.1 mg/kg haloperidol or 3.0 mg/kg clozapine. In every case, self-stimulation was sharply reduced in the dopamine deficient hemisphere but unaffected or enhanced in the dopamine intact hemisphere. Since this paradigm allowed each rat to serve as its own control the lateralized suppression in reward behavior could not be attributed to a motoric deficit. Futhermore, biochemical studies indicated that the neuroleptic drugs had a lateralized effect on dopamine turnover. In control rats, the neuroleptic drugs had variable effects on reward behavior ranging from slight to severe impairments. Significantly, when such rats were subsequently subjected to 6-hydroxydopamine lesions there was a strong positive correlation between the neuroleptic and 6-hydroxydopamine impairments in reward behavior. Thus, the combined treatment of neuroleptic drug and unilateral 6-hydroxydopamine lesion provides a powerful technique to demonstrate that dopamine has an important role in the reward aspect of brain stimulation reward behavior.

337.4 INTRACRANIAL SELF-STIMULATION OF THE NUCLEUS ACCUMBENS IN THE RAT: EFFECTS OF INTRACRANIAL INJECTIONS OF 6-HYDROXYDOPAMINE AT THE SITE OF STIMULATION. R.M. Clavier and D. Wen*. Biopsychology Research Section, Clarke Institute of Psychiat., 250 College St., Toronto, Canada M5T 1R8.

An electrode-cannula system was used to elicit intracranial self-stimulation (ICSS) from the nucleus accumbens in adult male albino rats in order to test the behavioral effects of local infusions of 6-hydroxydopamine (6-OHDA) into the brain area surrounding the electrode tip. ICSS responding was allowed to stabilize over a period of at least 10 days. 24 hours after the last ICSS trial, the animals were given injections of pargyline (Sigma; 50 mg/kg, i.p.), and 30 minutes later they were anaesthetized and placed into a stereotaxic apparatus. An injection cannula was then fitted into a guide cannula in the electrode system. An amount of 6-OHDA, ranging from 5 to 10 ug/2.5 ul (expressed as the base) dissolved in 0.15 M NaCl, and containing 0.2 mg/ml ascorbic acid, was injected over a period of 15 minutes. The injection cannula was left in place for 5 minutes after the infusion. Daily testing for ICSS at the same stimulation parameters as before the infusion resumed 24 hours after the infusion, and continued for at least 7 days. Again, 24 hours after the last of these sessions, a second infusion of 6-OHDA - similar to the first - was administered. The animals were then tested for ICSS on the following 14 days. Preliminary analysis of the behavioral data revealed that there was essentially no change in ICSS after the first injections, but that after the second injections there was an overall drop in bar-pressing, compared with baseline rates. The brains of these animals were subsequently prepared for examination of the stimulation area using the formaldehyde-induced fluorescence of catecholamines in Vibratome-sectioned material. Results of these anatomical, as well as of pharmacological studies currently under way, are presented.

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337.5 DOPAMINE RECEPTORS INVOLVED IN SELF-STIMULATION IN THE RAT. S. Nakajima. Department of Psychology, Dalhousie Univ., Halifax, Nova Scotia, Canada, B3H 4J1.

The suppression of self-stimulation produced by neuroleptic drugs has been attributed to the blocking of dopamine receptors in the brain. Since there are more than one type of dopamine receptor in the brain, a question was asked whether the suppression results from the blocking of either D1 (cAMP-mediated) or D2 receptors only, or blocking of both receptors together. Rats were implanted with bipolar electrodes into the lateral hypothalamic area, ventral tegmentum, dorsal raphe, or the septal area, and then trained to receive a 0.5 sec train of pulse stimulation by making a contact with a metal tube. To test drug effects, the animal was left to respond for 30 min prior to an i.p. injection and 60 min thereafter.

SCH-23390, a drug believed to be a specific blocker of D1 receptors, suppressed self-stimulation completely within 5-8 min of injection. As small as 0.05 mg/kg of SCH-23390 produced the same complete suppression as observed with 25 mg/kg haloperidol in all animals regardless of the electrode site . The animals were not asleep or paralyzed; there was no sign of pain or discomfort. Sulpiride, known to have a much higher affinity with D2 than D1 receptor sites, produced no effect on self-stimulation at 50 mg/kg. At 100 mg/kg, the rate of responding was reduced to 30-50% of the pre-injection rate, but none of the animals stopped responding completely. These animals were tested again 3 hours after injection for 30 min: all of them demonstrated about 50% level of responding.

The results suggest that the blocking of D1 receptors is sufficient to cause a complete suppression of self-stimulation in the rat. The effect of SCH-23390 cannot be attributed entirely to motor dysfunction because the task was extremely easy to perform. It is more likely that the brain stimulation was no longer rewarding when D1 receptors were blocked.

(Supported by NSERC of Canada, Grant No. A0233. Sulpiride was provided by Delagrange International, and SCH-23390 was a gift from Dr. G. M. McKenzie.)

337.6 DOPAMINE ANTAGONISTS PIMOZIDE AND SCH-23390 REDUCE HIPPOCAMPAL THETA-CONTINGENT SELF-STIMULATION WITHOUT IMPAIRING THE RAT'S ABILITY TO PERFORM THE RESPONSE. Bryan D. Fantie. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1.

Various pharmacological agents can increase or decrease the number of responses that an animal will produce in order to earn some reward. It is difficult to interpret these changes in performance as a direct measure of an alteration of the rewarding value of the reinforcer. For instance, a drug may suppress responding by interfering with motor activity even if it has no effect on the magnitude of the reward. This problem has proved particularly troublesome for studies dealing with rewarding intra-cranial self-stimulation. In order to overcome this difficulty, I have developed an experimental technique to help differentiate decrements in reward from performance deficits produced by motor impairment or decreased sensory capacity. Male hooded rats were implanted with bipolar electrodes from which hippocampal theta could be recorded. A second bipolar electrode, aimed at the lateral hypothalamic area of the medial forebrain bundle, was used to deliver rewarding electrical stimulation of the brain (ESB). In order to earn ESB the rat could hold a lever down for 3 s or, when the lever was retracted, produce a continuous 3 s train of hippocampal theta waves. Throughout each 3 hr session, the type of response required to produce ESB alternated every 5 min. Preliminary investigations have revealed that injections of SCH-23390 (0.05 mg/kg, i.p.), a drug reported to block D1 receptors specifically, or pimozide (0.5 mg/kg, i.p.) suppress both bar-pressing and rewarded hippocampal theta trains. Significantly, the unrewarded theta trains, which are produced when the lever is available, do not decrease thus demonstrating that the ability to produce hippocampal theta has not been impaired. Therefore, the decrease in the rate of rewarded theta trains produced by these drugs cannot be attributed to a performance deficit since the animal has demonstrated that it is still capable of performing the response.

(Supported by NSERC of Canada Grant No. A0233 to Dr S. Nakajima. SCH-23390 was kindly donated by Schering Corp. through the co-operation of Dr G.M. McKenzie.)

337.7 EFFECTS OF 6-HYDROXYDOPAMINE LESIONS OF THE MEDIAL PREFRONTAL CORTEX ON COCAINE SELF-ADMINISTRATION. C. Szostak*, M.T. Martin-Iverson and H.C. Fibiger (SPON: A. Jakubovic). Div. Neurol. Sci., Dept. Psychiat., University of British Columbia, Vancouver, B.C., V6T 1W5.

Destruction of dopamine (DA) nerve terminals or cell bodies in the nucleus accumbens (nA) results in a disruption of cocaine self-administration (CSA), suggesting that the nA plays a critical role in the reinforcing properties of cocaine (Roberts, D.C.S., et al., Pharmac. Biochem. & Behav., 12:781, 1980; Zito, K.A., et al., Neurosci. Abstr. 331.6, 1983). 6-Hydroxydopamine (6-OHDA) lesions of the ventral tegmental area (VTA) also attenuate CSA (Roberts, D.C.S. & Koob, G.F., Pharmac. Biochem. & Behav., 17:901, 1982). However, loss of DA in the nA following such lesions does not correlate with the observed behavioral deficits, suggesting that other structures innervated by the VTA may be involved in the maintenance of CSA. Accordingly, Goeder & Smith (Science, 221:773, 1983) reported that rats will respond for cocaine injected directly into the medial prefrontal cortex (MPFC) but not into the VTA or nA, suggesting that the mesocortical DA system may be involved in CSA.

To test this hypothesis, rats were implanted with chronic indwelling jugular cannulae and allowed to respond for cocaine (1.25 mg/ml; .18 ml/infusion) according to a discrete trial, continuous reinforcement schedule for 3 hrs/day. Once responding had stabilized, rats received either bilateral infusions of 6-OHDA (8 ug/2 ul) into the MPFC, or a sham operation. Testing for CSA recommenced 2 days post-operatively and continued until stable performance was obtained. HPLC analysis of DA and serotonin (5-HT) levels in the MPFC nA and striatum was conducted upon completion of behavioral testing.

Bilateral infusions of 6-OHDA into the MPFC reduced DA concentrations in this region to approximately 5% of control levels. 5-HT levels were not affected (96%). Despite the extensive depletion of DA, CSA was not disrupted, suggesting that the mesocortical projection is not essential for CSA. As such, responding for intra-MPFC infusions of cocaine may be mediated by the non-specific effects of cocaine (e.g. anaesthetic properties) rather than by direct effects on the DA nerve terminals. Alternatively, the mesocortical system, in conjunction with other systems may be involved in modulating the reinforcing properties of cocaine.

337.8 **PHARMACOLOGICAL DISSOCIATION OF THE CUE PROPERTIES FROM THE REINFORCING EFFECTS OF VENTRAL TEGMENTAL BRAIN STIMULATION.** **J.P. Druhan*, M.T. Martin-Iverson, D. Wilkie*, H.C. Fibiger and A.G. Phillips (SPON: R. Tees). Dept. Psychology; Div. Neurological Sciences, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.**

The reinforcing properties of electrical brain stimulation (EBS) at some sites appear to depend on the activation of dopaminergic neurons. By contrast, pharmacological treatments affecting dopamine systems do not alter the discriminability or the detection of EBS in the lateral hypothalamus, when the stimulation is used as a discriminative cue. The present experiment was designed to compare the effects haloperidol and amphetamine on both the reinforcing and cue properties of EBS delivered to a region containing dopamine cell bodies, the ventral tegmental area (VTA). Rats with VTA electrodes were trained to make a discriminated operant response on one of two levers after pulses of high or low intensity EBS, to obtain one food pellet. Ninety such trials were given in a daily session, with variable 20 sec intertrial intervals. Animals that learned the discrimination were subsequently given generalization tests in which intermediate current intensities were administered on 20% of the trials. Stimulus gereralization gradients were obtained for each animal under baseline conditions and following separate administrations of .075 mg/kg haloperidol and 2.0 mg/kg amphetamine. The effects of the same doses of these drugs on self-stimulation were subsequently determined during rate-intensity tests. Haloperidol and amphetamine did not alter the discrimination of EBS in the generalization procedure. In contrast, both drugs had significant effects on self-stimulation behavior. Amphetamine increased bar-press rates at each of the currents used in the discrimination procedure, while haloperidol decreased rates relative to those observed following vehicle injections. These effects support previous reports of dopaminergic involvement in the rewarding properties of VTA brain stimulation. However, the lack of an effect of these drugs on discrimination performance suggests that the cue properties of the EBS are not dependent on dopamine activity.

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337.9 **BEHAVIORAL AND BIOCHEMICAL STUDIES OF CENTRAL DOPAMINE RECEPTOR SENSITIVITY IN DIFFERENTIALLY HOUSED MICE.** **C.A. Wilmot, C. VanderWende, and M.T. Spoerlein. Dept. Pharmacology, Rutgers Univ., Piscataway, NJ 08854.**

The social isolation of male mice results in a syndrome characterized by hyperactivity in a novel environment, increased sensitivity to central stimulants, an impaired learning ability and a pronounced fighting behavior towards conspecific males and has been considered an animal model for psychoneurotic and learning disorders. Whether changes in the presynaptic regulation of dopaminergic neuron activity or in the postsynaptic sensitivity of dopamine receptors contribute to the behavioral differences between group-housed(GH) or individually-housed(IH) mice were examined. Previous work from this lab has shown an increased locomotor(LMA) response to low doses of amphetamine(AM) as well as an increased climbing response to low doses of apomorphine(APO) after 4 weeks of differential housing(DH).

To examine the hypothesis of an autoreceptor subsensitivity in IH mice, DH mice were compared in their LMA response to APO(0.0075-0.3 mg/kg) and in the APO-antagonism of GBL-induced DOPA accumulation after inhibition of aromatic amino-acid decarboxylase in striatal, limbic and frontal cortex tissue. Although IH mice show a significantly greater baseline LMA than GH mice, mice from both housing conditions were responsive to the activity-reducing effects of APO. Maximal effects were seen at APO 0.0375 and 0.075 mg/kg for IH and GH mice respectively. Also, there was no difference between GH and IH mice in the APO(.0075-2.0mg/kg)-antagonism of GBL-induced DOPA accumulation in the 3 brain regions examined.

The possibility of a postsynaptic hypersensitivity resulting from social isolation was also considered. The ability of APO(.075-0.6 mg/kg) to stimulate LMA after reserpine pretreatment (5mg/kg, 3.5 hrs.) was significantly greater in IH mice. Confirming this behavioral measure, 3H-Spiroperidol binding indicated a significantly greater number of high affinity sites in limbic tissue, a nonsignificant increase in frontal cortex and no significant change in striatal tissue, with no significant changes in Kd. These studies indicate that the behavioral syndrome associated with IH male mice may be concurrent with postsynaptic dopamine receptor hypersensitivity.

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337.10 BEHAVIORAL RESPONSES TO APOMORPHINE DIFFER IN NEONATAL AND ADULT-6-HYDROXYDOPAMINE-TREATED RATS. G.R. Breese, A.A. Baumeister, T.J. McCown, G.D. Frye, K.L. Hulebak and R.A. Mueller. Biological Sciences Research Center, UNC School of Medicine, Chapel Hill, NC 27514.

Central 6-hydroxydopamine(6-OHDA) treatment of adult and developing rats results in a major reduction of brain catecholamines. Recently, it has been observed that 100 mg/kg of L-DOPA induces self-biting in adult rats treated neonatally with 6-OHDA, but not in those animals treated as adults. The purpose of the present experiments was to determine if behavioral responses to apomorphine would also differ in neonatal- and adult-6-OHDA-treated rats. When neonatal-6-OHDA-treated rats were challenged with apomorphine (1 mg/kg) as adults, they exhibited stereotypies and self-biting. Adult-6-OHDA-treated rats did not exhibit self-biting, but they did display paw treading and head nodding - behaviors not observed in neonatal-6-OHDA-treated rats. In addition, the locomotor response to apomorphine (1 mg/kg) was significantly greater in adult-6-OHDA-treated rats than those treated neonatally. Brain dopamine was markedly reduced in striatum, nucleus accumbens, and olfactory tubercles in both treatment groups. Serotonin was elevated in the striatum of rats treated neonatally with 6-OHDA but not in adult-6-OHDA-treated rats. However, we could not attribute the behavioral changes after apomorphine administration to release of serotonin in the neonatal-6-OHDA-treated rats, because 5-hydroxytryptophan did not induce these behaviors when administered to these animals. To assess the role of dopamine receptors in the self-biting observed to dopamine agonists, neonatal-6-OHDA-treated rats were treated with flupenthixol or haloperidol before receiving L-DOPA. While administration of haloperidol did not reduce the incidence of self-biting, flupenthixol blocked this response elicited by this dopamine agonist. These data suggest that this behavioral response is associated with D-1 receptors. The dependence of the behavioral responses induced by dopamine agonists on the age at which dopamine-containing fibers are destroyed suggests that adaptive neural mechanisms (initiated by disrupting dopamine-containing fibers) differ in neonate and adult rats. (Supported by HD-03310 and MH-36294)

337.11 REPETITIVE, CONDITIONED RESPONDING FOLLOWING TELENCEPHALIC CATECHOLAMINE IMBALANCE: WHOLE BRAIN AND RESTRICTED LOCUS COERULEUS LESIONS. I.J. Goodman, A. Osman* & A.J. Azzaro. Depts. of Psychology and Neurology, West Virginia University, Morgantown, WV 26506

Repetitive, conditioned key pecking, promoted by fixed interval food delivery, is reported to drop following telencephalic dopamine (DA) depletion. This may be produced by the destruction of avian paleostriatal complex (basal ganglia) or the ventral midbrain (nucleus tegmenti pedunculo pontinus, substantia nigra homologue, and the ventral area of Tsai). On the other hand, a conditioned response rate rise is produced when a decrease in both DA and norepinephrine (NE) follows such a lesion. The present study investigated the interactive telencephalic DA-NE effects with lesions at other brain loci, in locus coeruleus (LoC) and from injections into cisternum magnum.

Intact pigeons, taught to key peck for grain reinforcement, responded on a schedule that had reinforcement delivered after the first response following a 2 min no-reinforcement interval (FI-2). Following the establishment of stable response rates and patterns, birds were bilaterally lesioned in LoC by electrolysis (2 mA, dc anodal current, 15 sec), 6-hydroxydopamine (6-OHDA)(8ug/2uL saline), pretreatment with desmethylimipramine (DMI)(15 mg/kg, ip) followed by 6-OHDA or pretreatment with bupropion (BPN)(40 mg/kg, ip) followed by 6-HDA. Other birds received intracisternal injections of 6-OHDA (200 mg/20 uL saline), preceded either by DMI or BPN. Birds were run an additional 30 or more daily behavioral sessions. Animals were sacrificed and high performance liquid chromatography assays were run for telencephalic DA and NE levels.

For purposes of comparing repetitive, operant responding and brain CA levels, two scores were used. The mean daily pecking frequency over the last 5 day block was the behavioral score and the CA score used was DA - NE/ NE/DA. These scores were determined for each subject. A consistent covariation between mean pecking frequency and the CA index was found; birds with highest pecking rates had the highest CA scores (highest ratio of DA to NE) and those with the lowest pecking rates had the lowest CA scores. This was independent of the site of lesion initiation. These results are consistent with reports suggesting an important interaction between telencephalic DA and NE that underlies repetitive responding in operant and stereotyping circumstances.

337.12 IMPULSIVITY AND ATTENTION DEFICIT IN RATS. G.K. Hodge, R.D. Becenti*, A. Kadashaw-Kelber*, A.E. Butt*, J. Salinas*, R.M. DeBoo* and C.R. Friedli*. Dept. of Psychology, Univ. of New Mexico, Albuquerque, NM 87131.

Attention deficit disorder is characterized by impulsivity and inattention (DSM-III, 1980). 6-Hydroxydopamine (6-OHDA) was used to model the disorder. Following desipramine pretreatment, 5-day-old rats were injected intracisternally with either 100 µg/25 µl of 6-OHDA or 25 µl of the ascorbic acid/saline vehicle.

Impulsivity was assessed by using a modification of a DRL task used by Gordon (J. Abnorm. Child Psychol., 7:317, 1979) to discriminate between hyperactive and nonhyperactive boys. Details of our procedure are described elsewhere (Hodge et al., Neurosci. Abstr., 9:553, 1983); briefly, the pups were required to delay approaching the dam until an overhead light came on. 6-OHDA impaired delay of responding between Days 19 and 26 postpartum. As with children treated with d-amphetamine, the drug (1.0 mg/kg) attenuated impulsivity in this animal model of the disorder.

Long term effects of 6-OHDA treatment on attention were assessed with a modified Kornetsky procedure (Psychopharmacologia, 8:277, 1965). Training began on Day 64 (at which time treated rats weighed less than controls). Rats maintained at 80% body weight were required to discriminate between critical and noncritical lights. Attention was operationalized as ommission errors (failure to respond to the critical light), impulsivity as commission errors (responses to noncritical light), and learning as successes (responses to critical light). There were no group differences in nondrug performance between Days 64 and 361. Random-ordered injections of saline or d-amphetamine (0.25, 0.5, 1.0, 3.0, 5.0 mg/kg) were initiated on Day 289. Compared to saline, no differences were produced by the two lowest doses. At the two highest doses, both groups failed to engage in goal-directed behavior (i.e., maximum ommission errors, no commission errors, no successes). This same lack of responding was seen in the 6-OHDA group at the 1.0 mg/kg dose, although the controls were unaffected by this dose. One explanation is that the 6-OHDA produced receptor supersensitivity; the 1.0 mg/kg dose disrupted behavior to the same extent produced in controls by higher doses. However, group sizes were small (6-OHDA, 2; controls, 3), and inclusion of two additional 6-OHDA-treated rats of different ages and experience failed to preserve this difference.

(Supported by DHHS grant RR08139)

337.13 EFFECTS OF SENSORY STIMULATION ON THE ACTIVITY OF DOPAMINE-CONTAINING SUBSTANTIA NIGRA NEURONS IN FREELY MOVING CATS. D.W. Preussler and M.E. Trulson. Dept. of Pharmacol., Marshall Univ. Sch. of Med., Huntington, WV 25701.

Dopamine (DA)-containing neurons in the substantia nigra have been implicated in a wide variety of sensory processes. The hypotheses that DA neurons play a role in sensory processes derive largely from single unit studies in anesthetized rats showing that these neurons are responsive to a variety of sensory stimuli. Since we have found that monoaminergic neurons respond differently in anesthetized vs. unanesthetized. animals, we tested a variety of sensory stimuli on the activity of DA containing neurons in awake, behaving cats. Unit activity was recorded by means of movable 32 and 64µ dia. Nichrome wires The following stimuli were tested: Noxious, an alligator clip was placed on the tail of the cat to apply moderate pressure; Tactile, all aspects of the cat's body surface were stimulated gently with a rubber probe; Olfactory, a stream of ammonium hydroxide saturated air was delivered throught a hole in the side of the recording chamber (the stimulus was sufficiently strong to cause the cat to withdraw and groom the nose with the forepaws); Auditory six clicks (110 db measured at the center of the recording chamber, 1 msec pulses) delivered at a rate of one click every 30 sec through a speaker ip the chamber wall; Visual, six light flashes (1.5×10^{0} candle power) delivered through a clear plexiglass window at the rate of one flash every 30 sec by means of a photic stimulator. There was no overall significant effect of any of the 5 types of sensory stimulation tested on the activity of DA units; 5/46 cells tested showed a significant change in their activity with noxious stimulation (+23-+34%); 2/47 cells responded to tactile stimulation (+12-+17%); 4/36 cells responded to olfactory stimulation (+17-+20%); 6/49 cells showed a significant response to auditory stimulation (+11-+14%); and 3/48 cells showed a significant change in response to visual stimuli (+10-+17%). These data are in contrast with those of other investigators, who have reported that noxious, tactile, olfactory, auditory and visual stimuli have a pronounced effect on DA cells in anesthetized rats. The difference may be due to the use of anesthesia in previous studies, since we have recently demonstrated that anesthesia alters the responsiveness of other monoamine neurons to sensory stimulation.

337.14 INTRANIGRAL INJECTIONS OF DOPAMINE AGONISTS AND ANTAGONISTS, GLYCINE AND MUSCIMOL INFLUENCE LOCOMOTOR ACTIVITY. E.A. Jackson and P.H. Kelly. Preclinical Research, Sandoz Ltd., CH-4002 Basel, Switzerland.

Previously we have shown that bilateral intranigral injections of dopamine into rats pretreated with a monoamine oxidase inhibitor induce prolonged stimulation of locomotor activity, while bilateral intranigral injections of haloperidol reduce the locomotor stimulation evoked by systemic amphetamine (Brain Research, 1983, 278, 366-369). These results suggest that dendritic dopamine release in the substantia nigra may have a functional role in locomotor activity. In the present studies, the role of the substantia nigra in locomotor activity was further investigated using intranigral injections of a variety of dopaminergic and other agonists and neuroleptics. Ergometrine, epinine, (±)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronapthalene hydrobromide (ADTN), 1,2,3,4-tetrahydro-6,7-dihydroxyisoquinoline hydrochloride (THIQ), muscimol and glycine elicited locomotor activity when injected into the substantia nigra pars reticulata bilaterally. Ergometrine was much less effective when injected into the reticular formation immediately above the substantia nigra. Additionally, intranigral injections of glycine or muscimol elicited a degree of stereotyped behavior in contrast to the effects of intranigral dopamine agonists. Locomotor activity induced by intranigral ergometrine was blocked by systemic haloperidol but was not affected by intranigral haloperidol. Locomotor activity elicited by systemic amphetamine was blocked by bilateral intranigral α-flupenthixol, but that elicited by bilateral intra-accumbens ergometrine was not affected by α-flupenthixol or haloperidol injected into the substantia nigra pars reticulata bilaterally. The results provide further evidence that alterations of neurotransmission in the substantia nigra exert effects on locomotor activity.

337.15 INCREASED NIGROSTRIATAL DOPAMINE TURNOVER IS ASSOCIATED WITH BEHAVIORAL ACTIVATION IN NEONATAL RAT PUPS. T. H. Moran*, K. M. Saad*, R. G. Robinson and E. M. Blass. (Spon: J. Wirth). Dept. of Psychiatry, Johns Hopkins Univ. Sch. of Med., Balt., MD 21205.

In response to a variety of experimental conditions, neonatal pups are able to exhibit a high degree of behavioral complexity. These demonstrations often require the environmental supports of high ambient temperature and some form of external stimulation, resulting in behavioral activation on the part of the neonate. Recent work has also indicated that function within the nigrostriatal dopamine (DA) system is dependent upon high ambient temperature. In order to determine whether increased function within this pathway was common to situations which are behaviorally activating to neonatal rat pups, we examined DA turnover in 3 day old rat pups in a variety of testing situations.

In an initial study, we examined the effect of high ambient temperature on striatal dopamine turnover by pretreating pups with AMPT and measuring striatal DA levels 0, 30, 60 and 180 minutes later in pups kept either at room (24° C) or nest (33° C) temperature. Following AMPT pretreatment, there was a progressive decline in striatal DA concentration over time only in pups kept at nest temperature ($F(3,27)=8.278$, $p<.001$). Mimicking the behavior of the dam, activating the pups by stroking them with a soft artist brush for 10 minutes resulted in significantly lower striatal DA concentrations following AMPT pretreatment ($t=2.538$, $p<.025$) and significantly higher DOPAC/DA ratios in pups without AMPT pretreatment ($t=2.487$, $p<.025$). Stroked pups also had significantly higher levels of behavioral activity ($t=15.56$, $p<.001$). In a third experiment, pups were allowed the opportunity for independent ingestion of milk. Pups were tested following an 8 hr deprivation for 10 minutes. Striatal DOPAC/DA ratios were significantly higher in pups with the opportunity for milk ingestion than littermate controls ($t=2.578$, $p<.025$) and the amount ingested was correlated with DOPAC/DA levels ($r=.67$, $p<.05$).

These results demonstrate that a variety of stimuli and testing situations which result in behavioral activation in neonatal rat pups increase DA turnover within the nigrostriatal DA system. This suggests a role for this system in mediating activation induced behavioral complexity in neonatal pups.

337.16 An Ethological Approach to Apomorphine and Haloperidol Induced Dyskinesias in the Guinea Pig. G. Gerstner*, K. Bellman and L.J. Goldberg. Depts. of Oral Biology, Kinesiology, Anatomy, and Crump Institute for Medical Engineering, UCLA, L.A., CA 90024.

The purpose of this study was to analyze the effect of oral dyskinesias on normal behavioral functioning of the guinea pig in a rich environmental setting and to conduct a detailed analysis of the ways in which their oral behaviors change as a result of drug-induced dyskinesias and stereotypies. A behavioral ethogram, including oral behaviors such as feeding, drinking, grooming, gnawing, etc. was scored from videotapes for all animals under a variety of normal and drug conditions. Detailed analysis of the oral patterns included, among others, measures of the burst-pause chewing pattern seen in normal animals, chewing rate, chewing efficiency and stereotypy. Ten animals were administered haloperidol daily on weekdays; 0.5 mg/kg i.m. for either 10-12 weeks or for 3-4 weeks. Several saline controls were also run. Some animals, additionally, were administered a single dose of apomorphine, 0.5 mg/kg i.m., prior to any drug treatment and then again after being withdrawn from haloperidol for 6-7 days.

Animals given haloperidol for either 3 or 10 weeks and then withdrawn alter their oral behaviors in a number of ways. One change is that the normal burst-pause chewing pattern seen while eating pellets is disrupted ($p<.001$, χ^2, difference in distribution of number of chews per burst). Two different burst-pause patterns emerged. Some animals chewed with unusually long bursts (e.g., 60-80 continuous chews rather than the normal average of 6-8) while others paused every 2 or 3 chews. In both cases the "efficiency" of feeding appeared to decrease when total pellets eaten, number of bursts per pellet and rate of pellet eating were considered. The rate of chewing, however, (≈6 Hz) appears unaltered. On the other hand, both control and haloperidol treated animals showed an extreme stereotypic behavior when given a single dose of apomorphine. All animals spent 72-100% of the total session doing nothing but an abbreviated lipping and mouthing activity, most like exploratory behavior. This activity is different from normal gnawing, eating and exploring in its slow rate (1-3 Hz), its rare application to any object and its preemption of orienting and all other functions.

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337.17 LOW LEVELS OF HALOPERIDOL INCREASE SUCROSE CONSUMPTION IN RATS. D. Deupree* and S. Hsiao. Department of Psychology, University of Arizona, Tucson, Az 85721

Dopamine activity has been shown to be correlated with the brain mechanisms of positive reinforcement of behavior. Dopamine receptor blockers, such as haloperidol, pimozide, and other neuroleptics, produce reductions in response rates of behaviors associated with positive reinforcement (barpressing for food, water, and reinforcing electrical brain stimulation). Thus, it has been proposed that neuroleptics may affect the reinforcing properties of stimuli. In the present study water deprived rats previously trained to drink from graduated burets were given access to a 10% sucrose solution, after first being injected with haloperidol (0.05, 0.10, or 0.20 mg/Kg i.p.) or saline. The rats given 0.05 and 0.10 mg/Kg haloperidol drank more of the sucrose solution than those given saline, while those given 0.20 mg/Kg haloperidol did not differ from the saline rats in sucrose consumption. In a separate experiment it was found that these same doses of haloperidol did not affect water consumption, indicating that the effect of haloperidol upon sucrose consumption seen in the first experiment was not due to a motor effect. These results could provide behavioral support for pharmacological studies which indicate that at low dose levels haloperidol may actually act to increase the activity of dopamine due to selective blockade of pre-synaptic dopamine autoreceptors.

338.1 SEROTONERGIC CONTROL OF AGGRESSIVE "STATE" BY THE ACCUMBENS/PREOPTIC AREA (APOA) 1. A ROLE FOR UPTAKE? A.I. Barkai*, M. Potegal, N. Sharpless, L.T. Kremzner* and S. Kowalik*. N.Y. State Psychiatric Inst., Albert Einstein College of Medicine, Columbia College of Physicians and Surgeons, N.Y.

"Priming" a female hamster by allowing it one biting attack on a drug-treated target hamster reduces the latency of the subsequent attack. "Satiating" a hamster with a series of targets until it meets a criterion of 3 successive target presentations without attack increases the latency and reduces the number of subsequent attacks. These behaviorally specific shifts in aggressive state generalize to naturalistic encounters between undrugged subjects (Potegal and tenBrink, JCP, 1984, 98, 66).

The search for the neurochemical bases of these effects has revealed that ^{14}C-5HT uptake is reduced 6% below control levels in APOA from attack-primed subjects while being increased 14% above control levels in attack-satiated subjects (see Table). The difference in uptake between primed and satiated subjects matched for time of sacrifice is highly significant [$t(14)=4.5$, $p<.005$]. Woolf-Augustinsson-Hofstee plots of uptake vs ^{14}C-5HT concentration show that the behaviorally-induced shifts in uptake are due to changes in V_{max} [$F(1,6)=7.86$, $p<.035$] but not K_m. The neurochemical specificity of these results is indicated by the absence of between group differences in APOA ^{3}H-GABA uptake V_{max} (Table shows values for highest substrate concentration) or in tissue levels of fluorometrically-determined GABA (Kremzner et al, Adv. Neurol.,1979) or radioenzymatically-determined norepinephrine (NE) or dopamine (DA)(Sharpless and Brown, Brain Res., 1978, 140, 171; see Table).

	5HT uptake[1] pmol/mg/5min	GABA uptake[2] pmol/mg/5min	GABA µmol/g	NE ng/g	DA ng/g
N	45	24	13	20	20
Priming	47.1±2.3	723±136	1.87±.03	1672±153	1838±238
Satiation	57.2±2.2	777±125	1.80±.23	1684±198	1558±362
Control	50.1±3.5	860±172	1.60±.44	1897±128	1459±317

values=mean±s.e.m. substrates= 2 µM 5HT[1], 100 µM GABA[2]

The absence of priming or satiation-induced uptake changes in frontal cortex or hippocampus, together with the failure of locomotor activity alone to alter APOA ^{14}C-5HT uptake suggests that aggressive state may be specifically regulated by changes in serotonin uptake local to the APOA.

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338.2 SEROTONERGIC CONTROL OF AGGRESSIVE "STATE" BY THE ACCUMBENS/PREOPTIC AREA (APOA) 2. DIFFERENTIAL EFFECTS OF 5,7-DHT INJECTED INTO LATERAL AND MEDIAL APOA. M. Potegal, A.I. Barkai*, L. Skaredoff* and J. Popken* (SPON: H. Mahut). New York State Psychiatric Institute, New York, N.Y. 10032

The serotonin neurotoxin 5,7-DHT was injected into lateral and medial APOA to determine if the uptake changes reported in the preceding abstract reflect these areas' serotonergic control of aggression. Individually housed, ovariectomized hamsters were screened for aggression with drug-treated target hamsters during the 10 hr dark phase of the reversed light cycle. Animals from 6 triplets matched for screening score were assigned equally among medial (M), lateral (L) and control (C) groups. One hr after DMI pretreatment (25 mg /kg ip) M subjects received a single midline POA injection of 10 µg 5,7-DHT in 2 µl cold saline, L subjects received bilateral 1 µl injections just lateral to the lateral POA, and C subjects received saline. On postinjection days 4, 9 and 14 a combined priming/satiation test was given: Immediately following an attack on an initial "priming" target, that target was replaced by an "exposure" target for 1 hr. A novel "switch" target was then alternated with the exposure target for 2 min periods until the satiation criterion of 3 consecutive alternations without an attack was met. A novel "probe" target was then introduced for the final 10 min.

HPLC-determined 5HIAA levels in M (40±44 ng/g) and L (34± 55 ng/g) group APOAs were significantly below C levels (191± 93 ng/g)[$F(2,15)=10.5$, $p<.001$]. DOPAC and HVA were unaffected. Lateral 5,7-DHT reduced aggression, medial 5,7-DHT increased it: Mean attack latencies of L, C and M groups were 5.8, 3.8 and 2.6 min, respectively. Mean number of attacks on alternation and probe targets were 0.7, 4.3 and 4.6 for L, C and M groups, respectively (for L vs M attack number, $t(5)=3.54$, $p<.017$, which is significant even with Bonferroni adjustment of α for multiple tests). The increase in attack latency and decrease in attack number in the L group resembles the changes following attack satiation. This shift in aggressive state may therefore involve a reduction in lateral APOA serotonergic activity, possibly mediated by increased serotonin uptake. The M group's pattern of reduced attack latency accompanied by only a slight increase in attack number resembles that following attack priming. Changes in medial APOA serotonergic activity may be involved in attack priming.

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338.3 PONTO-GENICULO-OCCIPITAL (PGO) WAVES: PARAMETRIC STUDIES OF THEIR TEMPORAL DISTRIBUTION IN RELATION TO THE SLEEP CYCLE AND DORSAL RAPHE (DRN) DISCHARGE. R. Lydic, R.W. McCarley and J.A. Hobson. Lab. of Neurophysiology, Harvard Medical School, Boston, MA 02115

Although the neural substrates underlying both desynchronized (D) sleep and PGO waves were localized to the pons well before 1960, the synaptic mechanisms of PGO wave generation are presently unclear. Evidence suggests that PGO waves result from an interaction between: (1) putative generator cells with output neurons located in the peribrachial region of the anterodorsal pons; and (2) a mechanism disinhibitory to PGO waves mediated, in part, by the cessation of DRN discharge.

Recently, alterations in DRN and PGO wave activity were described as a function of sleep cycle duration (Tau) (Brain Res. 274: 365, 1983) and forced locomotor activity (Am. J. Physiol.In Press, 1984). To determine whether the temporal characteristics of PGO wave occurrence are compatible with the postulated DRN disinhibition, the present study characterized the PGO-DRN relationship for a large number (N=102) of sleep cycles without selection for Tau or behavioral preconditions. Each sleep cycle was operationally defined from D end to D end and cycles ranged from 9 to 73 mins. (mean Tau=28.8 mins). Analyses of PGO wave discharge frequency within discrete behavioral states revealed: Wake=0.01 waves/s, Synchronized sleep=0.04, Transition=0.35, and D sleep=0.59 PGO waves/s. To describe the average time-course of PGO wave activity, sleep cycle duration was expressed as percent cycle completed (0 to 100%). Less than 5% of the PGO waves occurred during the first third of the average cycle, 15% by mid-cycle, and 63% during the final third of the cycle (correlation (r)=+0.97). Regression analyses of PGO wave activity (Y) across percent cycle completed (X) revealed a statistically significant positive slope (2,7) with 94% of the variance in Y accounted for by the fitted regression. DRN discharge was negatively correlated (r=-0.93) with the PGO activity curve.

Thus, the average PGO and DRN activity profiles are inversely correlated across all sleep cycle Taus. These results are compatible with the hypothesis that PGO waves are modulated by DRN discharge.

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338.4 STUDIES OF THE SUBSTRATES OF THE HYPERACTIVITY PRODUCED BY ELECTROLYTIC LESIONS OF THE MEDIAN RAPHE NUCLEUS. D. Wirtshafter, W. Montana & K.E. Asin. Dept Psychology, Univ. Illinois at Chicago. Chicago, Il. 60680.

Many workers have shown that electrolytic lesions of the median raphe nucleus (MR) lead to a pronounced increase in activity in novel enviornments, but little is known as to the substrates of this effect. Studies using neurotoxins have shown that serotonin depletion is not the primary cause of the increased activity and that damage to non-serotonergic cells or fibers of passage must occur for hyperactivity to be produced. In the current study we examined the substrate of MR lesion-induced hyperactivity by studying locomotion after placements of knife cuts (made with a retractable tungsten knife) in the region of the MR.

Coronal knife cuts through the main ascending serotonin bundles at the anterior border of the MR produced large depletions of hippocampal and striatal serotonin but did not influence activity. The dorsal border of these cuts was at the central grey and the ventral border about 0.8mm dorsal to the medial lemnisci. More ventral cuts at the same AP level (extending dorsally 0.8mm from the lemnisci) produced only small changes in serotonin,tended to decrease open field activity, but produced small, significant, increases in tilt box activity. Coronal cuts through the MR at the level of the ventral tegmental nuclei led to large increases in open field and tilt box activity similar to those seen after electrolytic lesions and depleted hippocampal but not striatal serotonin. Coronal cuts just caudal to the MR failed to alter forebrain serotonin but did produce modest increases in open field and tilt box activity. Animals with combined cuts rostral and caudal to the MR displayed pronounced hyperactivity, similar to that of rats with electrolytic lesions, in both tests. Parasagittal cuts placed on either side of the MR failed to alter either activity or serotonin levels.

These results demonstrate that it is possible to dissociate changes in activity and forebrain serotonin with midbrain knife cuts. Since hyperactivity of the magnitude seen after electrolytic lesions could be produced by a combination of cuts in front of and behind the MR, the individual cuts having much smaller effects, one interpretation of the current results is that the hyperactivity produced by electrolytic lesions may reflect damage to two seperate systems, one of which passes through the anterior and the other the posterior border of the MR.

338.5 ROLE OF THE MIDBRAIN RAPHE NUCLEI IN THE ATTENUATION OF NEUROLEPTIC INDUCED CATALEPSY. A.Kozlowski, D. Wirtshafter, and K.E. Asin (SPON: J.D.Davis). Dept. of Psychology, Univ. of Illinois at Chicago, Chicago, Il 60680.

There have been a number of reports suggesting that lesions of the midbrain raphe nuclei are able to antagonize various actions of neuroleptic drugs and these results have been generally attributed to depletion of forebrain serotonin. The current study attempted to isolate the neuronal elements within the raphe nuclei responsible for the attenuation of haloperidol-induced catalepsy.

Rats were tested for catalepsy 30 and 60 minutes after injections of 0.15 mg/kg haloperidol (s.c.). In the first experiment, electrolytic lesions were placed in either the median (MR), the dorsal (DR), or both the median and dorsal raphe nuclei and catalepsy was examined four days later. The DR and MR+DR lesions produced equivalent reductions in catalepsy, while the MR lesions were without effect. Open field activity was measured several days later and it was found that MR lesions produced a large increase in locomotion while DR lesions resulted in a much smaller effect. Biochemical analysis showed that DR and MR lesions resulted in depletions of striatal and hippocampal serotonin, respectively, but the magnitude of these depletions did not correlate with either catalepsy or open field activity. Histological examination revealed that the dorsal lesions damaged both the dorsal raphe nucleus and adjacent portions of the ventral central gray.

In the second experiment, injections of either the serotonin neurotoxin 5,7-DHT or the excitotoxic agent ibotenic acid were made into the DR. Although these lesions produced striatal serotonin depletions equivalent to the electrolytic lesions, neither lesion produced any attenuation of haloperidol-induced catalepsy. Histological studies indicated that the ibotenic acid lesions were better confined to the DR than the electrolytic lesions studied earlier. In other studies we found that treatment with the serotonin depleting drug p-chloroamphetamine (10 mg/kg) 3 and 4 days before testing also fails to attenuate catalepsy.

These findings demonstrate that different mechanisms within the raphe nuclei are involved in the production of hyperactivity and the attenuation of catalepsy. Furthermore, our results indicate that antagonism of catalepsy after DR lesions is not secondary to serotonin depletion but may reflect damage to fibers of passage within the area of the DR or nonserotonergic cells located adjacent to it.

338.6 CONCURRENT 5HT-1 AGONIST AND 5HT-2 ANTAGONIST ACTIVITY OF LISURIDE. R.Gerber, B.S.Barbaz, L.L.Martin* and J.M.Liebman. Neuroscience Res., Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

Lisuride, an ergoline derivative, possesses serotonin (5HT) agonist activity in neurochemical and behavioral assays (White & Appel, Science 216:535, 1982). This substance has other properties, such as dopamine agonism, and particularly noteworthy, a lack of hallucinogenic activity in man. The present experiments suggest that lisuride may have a potentially novel profile in behavioral assays of 5HT receptor-mediated activity.

In confirmation of previous studies (Silbergeld & Hruska, Psychopharamcology 65:233, 1979), lisuride potently induced the 5HT-syndrome in rats (ED_{50} = 0.36 mg/kg s.c.). Surprisingly, however, lisuride antagonized 5HTP-induced head twitches in rats (ED_{50} = 0.05 mg/kg i.p.). This 5HT antagonistic activity of lisuride was apparent at doses lower than those that caused a 5HT-syndrome. A number of other putative 5HT agonists, such as 8-hydroxy-2-(di-n-propylamino)-tetralin, 5-methoxy-N,N-dimethyltryptamine, quipazine and 6-chloro-2-(1-piperazinyl)-pyrazine all potently produced the 5HT-syndrome but, unlike lisuride, did not antagonize 5HTP-induced head twitches. Binding assays also suggested an unusual profile for lisuride. The ability of lisuride to displace rat cortex ^{3}H-5HT binding was confirmed (IC_{50} = 4.0 nM), but lisuride also displaced ^{3}H-ketanserin binding (IC_{50} = 6.3 nM). Other 5HT agonists showed good displacement of ^{3}H-5HT binding to rat cortex, but had little or no ability to displace ^{3}H-ketanserin binding. Apomorphine and bromocriptine did not induce the 5HT-syndrome nor antagonize 5HTP-induced head twitches, indicating that dopamine agonism cannot account for the observed effects of lisuride.

These results show it is possible for a drug to induce the 5HT-syndrome, yet block 5HTP-induced head twitches. They are consistent with the suggestion (Lucki et al., J. Pharmacol. Exp. Ther. 228:133, 1984) that the 5HT-syndrome is mediated by 5HT-1 receptors and the 5HTP-induced head twitches by 5HT-2 receptors. The apparent serotonin antagonist activity of lisuride may be related to its reported lack of hallucinogenic activity.

338.7 5-HYDROXYTRYPTOPHAN (5-HTP)-INDUCED HEAD-TWITCHING IN MICE: ANTAGONISM BY 5-HT_2 RECEPTOR ANTAGONISTS. Jeffrey B. Malick and Evelynjeane B. Sutton, Biomedical Research Dept., Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, DE 19897.

Recent evidence has demonstrated the existence of more than one population of serotonin (5-HT) receptor sites in the brain; the two populations identified to date have been designated 5-HT_1 and 5-HT_2. These types of receptors can be clearly distinguished by radiolabelled ligand binding assays but few studies have used behavioral models to characterize these sites. Recently, agents have become available that show selective antagonism at 5-HT_2 receptor sites (i.e., only very weakly active at 5-HT_1 receptors). The goal of the present study was to compare the effects of selected 5-HT antagonists with varying affinities at the two sites on 5-HTP induced head-twitching in mice, a procedure commonly used to assess antiserotonergic activity in vivo (Carne et al., Br. J. Pharmacol. 20: 106, 1963). Agents that have been reported to be potent and selective antagonists at 5-HT_2 receptors (e.g., pirenperone, pipamperone, ketanserin) were shown to be more potent as head-twitch antagonists than the less selective serotonin antagonists (e.g., cinanserin, methysergide). Thus, the 5-HTP induced head-twitch response in mice appears to be mediated at least in part by activation of 5-HT_2 receptor sites. Therefore, this procedure may be useful for assessing 5-HT_2 antagonist activity in vivo.

338.8 ANTICONFLICT EFFECT OF THE PUTATIVE SEROTONIN RECEPTOR AGONIST 8-OH-DPAT. J.A. Engel, S. Hjorth*, K. Svensson*, A. Carlsson* and S. Liljequist. Dpt. Pharmacol., Univ. of Göteborg, Box 33031, S-400 33 Göteborg, Sweden.

Accumulating evidence from animal experiments implicate the serotonin neurons in the control of behavior suppressed by aversive stimulation. Thus, drugs that interfere with the 5-hydroxytryptamine (5-HT) system, have been shown to produce a release of behavioral suppression. 8-Hydroxy-2-(di-n-propylamino)tetralin(8-OH-DPAT) was recently characterized as a centrally acting 5-HT agonist (Hjorth et al., 1982). This prompted us to investigate the effect of 8-OH-DPAT on behavioral suppression, using a modified Vogel's conflict paradigm.

Administration of 8-OH-DPAT produced an increase in the number of shocks accepted. This anticonflict effect of the putative 5-HT-agonist 8-OH-DPAT was unexpected since previous reports have shown that manipulations known to reduce 5-HT neurotransmission can produce anticonflict effects whereas tryptaminergic agonists tend to suppress behavior below control levels. In agreement with those reports we found that pretreatment with the 5-HT synthesis inhibitor PCPA for three days produced a significant increase in the number of accepted shocks. 8-OH-DPAT antagonized the PCPA-induced release of punished behavior, in fact to such an extent that the number of accepted shocks was below that of saline-treated controls. Thus in naive animals 8-OH-DPAT, excerting anticonflict effects, acted like a 5-HT-antagonist whereas in subchronically PCPA-pretreated animals with presumably supersensitive 5-HT receptors 8-OH-DPAT, decreasing the number of accepted shocks, acted like a 5-HT-agonist.

Taken together these data indicate that in the present behavioral paradigm 8-OH-DPAT acts like a partial 5-HT-agonist with a low degree of intrinsic activity, which is unmasked when the 5-HT-receptors are made supersensitive. Thus these data provide further support for the hypothesis that the intrinsic activity of a partial agonist may be related to the adaptive state of the receptor (Carlsson, 1983).

338.9 BRAIN MONOAMINE CHANGES WITH INESCAPABLE SHOCK. D.J. Anderson*, J.O. Johnson, P.T. Leyra*, F.A. Henn. Department of Psychiatry and Behavioral Science, SUNY at Stony Brook, Stony Brook, NY 11794

The effects of inescapable shock on brain monoamine levels in six regions of rat brain were studied using HPLC/EC. We have previously reported an increase in beta-adrenergic receptor density in the hippocampi of rats which have developed "learned helplessness". (ASN Abst. 340.9, 1983). Others have reported changes in monoamine levels using the triadic yoked control paradigm (Hellhammer, et. al., ASN Abst. 162.1, 1983). The present study assessed the possibility that up-regulation of beta-adrenergic receptors may be related to changes in monoamine levels.

Male, adult rats were subjected to randomized, uncontrollable foot shock for forty minutes (0.8mA). Twenty-four hours after the training session these rats were tested under the same conditions except escape by bar press was presented for 15 trials. The response deficient group failed to escape 11-15 times, while the Low group had 0-5 failures. A control group was formed by rats which were tested but had no inescapable stressor. A naive group was neither trained nor tested.

Two to four hours after the testing session, the rats were sacrified by decapitation and the brains dissected. The locus coeruleus, septum, thalamus, anterior neocortex, hippocampus and hypothalamlus were sonicated in 0.2M perchloric acid, centrifuged, and stored at -70^{0}C. NE, EPI, DOPAC, DA, 5-HIAA and 5-HT levels were determined for each brain area. We found no significant changes in NE levels in any of the brain areas. When NE/5-HT ratios are compared, significant differences between Highs and Lows are found in the hypothalamus ($p<.01$), the locus coeruleus ($p<.01$) and the anterior neocortex ($p<.05$). The susceptible group, whose behavior resembles depression, had ratios lower than control and naive groups, while the resistant group had higher ratios. This pattern was found in all brain areas investigated except thalamus. These acute effects to mild shock do not reflect changes seen in beta-adrenergic receptors using this paradigm indicating other postsynaptic factors are regulating this receptor under these conditions. However, these data indicate that an imbalance in NE- 5-HT levels may be involved in producing the effects seen in the learned-helplessness model of depression.

338.10 EFFECTS OF p-CHLOROPHENYLALANINE ON LEARNED HELPLESSNESS IN THE RAT. E. Edwards, J. Johnson, D. Anderson, P. Leyra and F.A. Henn. Dept. of Psychiatry and Behavioral Science, SUNY at Stony Brook, NY 11794.

Recently, it has been proposed that stressors such as immobilization, fighting and shock affect the levels and turnover of serotonin (5-HT). This suggests that serotonergic mechanisms may be involved in the mediation of the effects of inescapable shock on subsequent behavior.

Little is known, however, of the role of 5-HT in the development of escape deficits observed in the learned helplessness phenomenon. This study examines the effect of a potent 5-HT depletor, p-chlorophenylalanine (PCPA) on inescapable shock-induced deficits in rats subsequently tested with a shock escape paradigm.

PCPA was administered i.p on a three day course (3X 100mg/kg). Motor activity measurements were carried out 96 hrs. after the last PCPA injection prior to preshock treatment and also after the shock escape task. Five days after the last PCPA injection, the rats were pretreated with random shocks (.8mA) for forty minutes and then tested on a shock escape task 24 hours later. Four groups (N=20rats/group) were generated: PCPA-no shock (P-NS); PCPA-inescapable shock (P-IS), control saline-no shock (C-NS) and control saline inescapable shock (C-IS). After behavioral testing, the rats were sacrified and monoamine levels in the raphe, cortex, hippocampus, septum and hypothalamus were determined by HPLC/EC. ^{3}H-5HT and ^{3}H-spiroperidol binding were used to examine the status of the postsynaptic S_1 and S_2 receptors.

PCPA-treated rats, regardless of shock pre-treatment, demonstrated significantly less failures in shock escape testing than no drug and naive controls (3.8±1.7 failures, P-CS and P-IS vs 11.3±1.5 failures, helpless rats). This reduction in the learned helplessness effect was not due to the rats heightened reactivity. Motor activity indices (crossings, rearings) of PCPA treated rats paralleled that of controls (7.8±1.8; 6.55±1.5, PCPA vs 9.2±3.4; 4.0±2.2 controls).

Brain 5-HT/5HIAA levels were significantly reduced in PCPA treated rats as compared to controls. A 50% decrease was seen in the raphe, hypothalamus and septum while cortex and hippocampus exhibited up to 80% decrease in 5HT content as compared to controls. NE, EPI, DA levels were unchanged regardless of PCPA and shock treatment.

Scatchard analysis of ^{3}H-SPIP binding revealed no significant changes in the S_2 receptors of PCPA treated rats as compared to controls. Conversely, S_1 receptors demonstrated a significant degree of supersensitivity displayed as an increase in receptor affinity (Kd decreased significantly : P-CS and P-IS 7.35±1.05 nM vs C-NS and C-IS 12.0±1.0 nM NS and IS hippocampus; P-CS and P-IS 7.25±.25 nM vs C-NS and C-IS 12.87± 1.2 nM cortex).

The findings of the experiments outlined here support the interpretation of the learned helplessness phenomenon on the basis of mediation via the serotonergic system.

338.11 RAPHE HYPERACTIVITY: ATTENUATION WITH DEPLETION OF DOPAMINE IN THE CAUDATE AND NUCLEUS ACCUMBENS. L.L. Wing, K.E. Asin and D. Wirtshafter. Dept. of Psychology, University of Illinois at Chicago, Box 4348, Chicago, IL, 60680.

It has been well-established that electrolytic lesions of the median raphe nucleus produce hyperactivity. We have reported previously that depletion of forebrain dopamine (DA) with the neurotoxin 6-hydroxydopamine (6-OHDA) in rats inhibited the production of hyperactivity by electrolytic median raphe lesions given subsequently.

The purpose of the present study was to investigate if depletions of DA in the basal forebrain abolish the hyperactivity produced by prior electrolytic lesions of the median raphe (MR). We also examined the responses of these raphe-lesioned, DA-depleted animals to various doses of the DA agonist apomorphine (APO).

Thirty-three adult male Sprague Dawley rats served as subjects. Twenty-one were given a lesion of the MR nucleus by passing a 1 mA current for 8 seconds. Twelve other rats were sham-operated. One week post-operatively, activity was measured in an open field for 5 minutes and also in a tilt-box for 1 hour. Half of the sham-operated animals and 13 of MR lesioned rats were given bilateral infusions of 6-OHDA (4 ul; 6.5 ug/ul) into the anterior lateral hypothalamus, caudal to the accumbens. The rest of the sham-operated and MR lesioned animals were given infusions of the 0.1% ascorbate vehicle alone, thus resulting in 4 groups: Sham-Vehicle, Sham-6-OHDA, MR-Vehicle and MR-6-OHDA.

Twelve days following injection, all animals were tested again in the open field and one day later in the tilt-boxes. They were then run in the tilt-boxes on alternate days under the following conditions: saline; 1.5mg/kg amphetamine; 0.03 mg/kg APO; 0.12 mg/kg APO; 0.24 mg/kg APO; saline; and 0.06 mg/kg APO.

6-OHDA injections abolished the effect of MR lesions on open field activity and reduced, but did not completely eliminate the hyperactivity in the first post-operative tilt-box activity test. However, when tested two days later under saline, the hyperactivity appeared to be eliminated, and activity did not differ between MR-6-OHDA and Sham-6-OHDA groups. APO produced a dose dependent increase in activity in animals treated with 6-OHDA, the magnitude of which was not altered by previous raphe lesions.

These results are compatible with the notion that MR lesion-induced hyperactivity may result from alterations in DA turnover within the basal forebrain.

338.12 PATTERNS OF PLATELET SEROTONIN (5HT) UPTAKE AS INFLUENCED BY CATECHOLAMINES. R.F. Walker* and L. Humphries* (SPON. B. Peretz). Departments of Anatomy and Psychiatry, Univ. of Kentucky Medical Center, Lexington, KY 40536.

The ability of platelets to accumulate 5HT is often disturbed in patients with psychiatric illness. Although the mechanism by which mental state alters physiologic properties of platelets is unknown, the structures possess adrenoreceptors that could receive neural signals from sympathetic fibers innervating the vasculature. A similar relationship between the autonomic nervous system and β adrenergic receptors on pinealocytes is well established for regulation of serotonin metabolism. In the present study, we examined daily patterns of 5HT uptake in platelets from hospitalized psychiatric patients as a prelude to investigating possible functional interactions between platelet adrenoreceptors and the ability of these structures to accumulate 5HT. Controls selected from the hospital staff had one of two uptake patterns. A "symmetrical" pattern, statistically associated ($p<0.05$) with young subjects (<30 years) had increased uptake ($p<0.01$) at 1400h compared with 0900h or 1600h. Older controls (>35 years) had "ascending" patterns with increasing uptake from 0900h-1600h. Patterns were not linked to sex. Uptake was more variable in unmedicated psychiatric patients who had "inverted" or "descending" profiles in addition to the aforementioned patterns. Treatment with catecholamine neuroleptics normalized 5HT uptake in patients with certain diagnoses. When catecholamines were coincubated with platelets from unmedicated patients with these diagnoses, 5HT uptake was normalized in vitro. Since CNS catecholamine deficits and aberrant 5HT uptake occur in certain psychiatric illnesses, the findings of this study suggest that monoaminergic signals influence platelet 5HT uptake. Such signals may reach platelets adrenoreceptors via the sympathetic nervous system, thus providing an important link for communication between the brain and vascular structures. Supported by AG02867(RFW).

338.13 DIFFERENTIAL EFFECTS OF MAGNESIUM DEFICIENCY ON OFFENSIVE AND DEFENSIVE AGGRESSIVE BEHAVIOR. K.M. Kantak and B. Turnbull. Lab. of Behavioral Neuroscience, Dept. of Psychology, Boston University, Boston, MA 02215.

Previous research from our laboratory has demonstrated that limiting dietary magnesium (Mg) to 15% or 25% of the daily requirement leads to reductions in offensive threat and attack behavior in male mice. These changes were measured 3 weeks and 7 weeks after initiation of the various diets. Additionally, after 8 weeks on these diets, reductions in apomorphine-induced sniffing and l-amphetamine-induced locomotion were measured. In the present experiments, a weekly time-course of the effects of the 15% required-Mg diet or the 100% required- Mg control was established for offensive threat and attack behaviors in resident male mice and for defensive escape and upright posturing behaviors in intruder male mice. Concomitantly, a weekly time-course of the effects of the 15% or 100% required-Mg diets on apomorphine-induced sniffing, l-amphetamine-induced locomotion and quipazine-induced serotonin syndrome were examined to correlate the changes in agonistic behaviors with changes in dopamine, norepinephrine and serotonin function, respectively.

Results demonstrated that in resident mice, threat and attack behavior were decreased beginning 3 weeks after initiation of the 15% required-Mg diet and continuing through the 8th week of testing. In intruder mice, escape and upright posturing were increased beginning 5 weeks after initiation of the 15% required-Mg diet and continuing through the 8th week of testing. In addition, these changes in offensive and defensive behaviors occurred independently of general activity level and body weight level. The time course of changes in drug-induced behaviors showed differences among neurotransmitter systems. Reductions in norepinephrine and serotonin related behaviors were observed beginning after 1 week of exposure to the 15% required-Mg diet. In contrast, a reduction in dopamine related behavior was observed beginning after 5 weeks of exposure to the 15% required-Mg diet. These data indicate that offensive behavior and defensive behavior are differentially affected by a deficiency of magnesium in the diet. Furthermore, there are time differences to the onset of these effects. These differences may stem from the diverse time course changes in brain neurotransmitter functions with magnesium deficiency.

Supported by Boston University funds for Faculty Research.

338.14 PHARMACOLOGICAL EFFECTS OF MAGNESIUM ON AGGRESSIVE BEHAVIOR. S.E. Izenwasser and K.M. Kantak. Lab. of Behavioral Neuroscience, Dept. Psychology, Boston University, Boston, MA 02215.

Previously this laboratory has reported that the restriction of dietary magnesium to 15% or 25% of the daily requirement reduced offensive aggression in male mice (in the resident-intruder paradigm). Also reported were concomitant decreases in dopamine and norepinephrine functioning, measured by the amount of stereotypy seen following apomorphine and the amount of locomotion induced by l-amphetamine, respectively. In the present study, the effects of magnesium excess (resulting from chronic injections of magnesium chloride) on offensive behavior were examined.

After stable baselines of offensive behavior were achieved, male mice were injected daily with subcutaneous injections of either magnesium chloride (30 mg/kg, 60 mg/kg or 125 mg/kg) or saline (as a control) for two weeks. The mice were tested for offensive aggression five minutes post-injection on days 1, 4, 8 and 15. They were again tested on day 29, two weeks after the last injection.

Those mice receiving magnesium chloride showed dose-dependent increases in offensive threat and attack behaviors as compared to the saline controls following acute administration, with lower doses leading to greater increases in behavior than higher doses. Chronically, there were dose-related decreases in offensive behavior in that those animals receiving a daily dose of 125 mg/kg exhibited a decrease, whereas those receiving either 60 mg/kg or 30 mg/kg behaved at control levels. The amount of offensive behavior was equal for all groups on day 29, indicating that the chronic effect is reversible.

Thus, acutely administered, magnesium facilitates offensive aggression while chronically, higher doses decrease and lower doses have no effect on offensive behavior. These data and our previous data with magnesium deficiencies suggest an inverted U-shape function to magnesium's influence upon behavior. Additionally, since aggression has been linked to the neurotransmitters DA, NE and 5-HT and magnesium has been shown to be an important cofactor for the activity of these neurotransmitters, it is possible that the effects seen here are related to changes in one or more of these systems.

Supported by Boston University funds for Faculty Research.

MONOAMINES AND BEHAVIOR: ACETYLCHOLINE AND NOREPINEPHRINE

339.1 IN VIVO ELECTROCHEMICAL COMPARISONS OF THE EFFECTS OF CHRONIC INESCAPABLE SHOCK VERSUS ELECTROCONVULSIVE SHOCK TREATMENT IN RATS. C.W. Hughes and H.J. Pottinger*. Psychiat Dept, Univ Kans Sch Med, Kansas City, KS 66103.

Various inescapable (IS) and electroconvulsive shock (ECS) paradigms have been proposed as animal models of human neuropsychiatric disorders. Behavioral deficits from acute IS have been linked to central changes in monoaminergic systems. Acute ECS, as well, alters central monoamines. The central adjustments to chronic IS and ECS treatment have been postulated to be analogues to chronic ECT and pharmacologic treatment in humans. To assess monoamine differences between IS and ECS and the in vivo progression of monoamine responsivity over time, we measured changes in peak concentration of monoamines, duration of the responses, and regional variations with in vivo electrochemistry.

Male Sprague-Dawley rats weighing 250-370 gms were stereotaxically implanted in the anterior caudate (A.C.), nucleus accumbens (N.A.), and bed nucleus (B.N.) with carbon-epoxy paste working electrodes. A NOVA-3 minicomputer controlled chronoamperometric measurements. Four groups were tested for two weeks each: IS = daily grid shock, SHAM = IS procedure without shock, ECS = daily electroconvulsive treatment, and untreated. Each animal was monitored electrochemically every other day for two hours prior to and four hours following treatment. Data is presented for the initial exposure and 2 weeks after beginning treatment.

		Control n=12		SHAM n=6		IS n=6		ECS n=3	
	Day	Peak	Dur	Peak	Dur	Peak	Dur	Peak	Dur
N.A.	1	0	0	17	18	53	36	70	90
	14	0	0	17	12	60	240	147	198
A.C.	1	0	0	12	15	50	63	17	36
	14	13	12	40	63	80	240	112	240
B.N.	1	0	0	16	12	77	60	14	15
	14	0	0	--	--	113	240	42	98

Peak Change = micromolar conc. increase from baseline.
Duration = total minutes to return to baseline.

Untreated rats show no significant potentiation in response from baseline with chronic in vivo recording. The average increase in peak and duration from baseline for the other groups, respectively, were: SHAM = 100% and 233%; IS = 115% & 470%; and ECS = 400% & 470%. These changes in monoamine responsiveness appear to reflect both treatment differences as well as a progressive change in neurotransmitter availability with the greatest changes overtime reflected in ECS treatment.

339.2 SENSITIZATION OF NOREPINEPHRINE ACTIVITY FOLLOWING ACUTE AND CHRONIC STRESS. Jill Irwin and Hymie Anisman. Dept. Psychology, Carleton University, Ottawa, Canada.

Acute exposure to an uncontrollable stressor increases the turnover of brain norepinephrine (NE). With a stressor of sufficient severity, utilization may exceed synthesis, resulting in a net reduction of NE concentrations. Although the NE reduction is transient, subsequent reexposure to a limited amount of stress will provoke a rapid and marked decline of the transmitter. Thus it was suggested that the mechanisms subserving the stressor-induced NE alterations are subject to conditioning or sensitization processes. Contrary to the effects of acute stress, chronic stress engenders a compensatory increase of NE synthesis, and as a result, levels of the transmitter may meet or exceed those of nonstressed animals.

Several experiments evaluated NE activity upon stressor reexposure at brief (24 hr) and long (14 days) intervals following application of an acute or chronic stress regimen. In hypothalmus and hippocampus a marked increase of NE utilization (as measured by MHPG accumulation) was provoked by a limited amount of shock (which in itself had little effect) in mice that were previously exposed to an acute stress session. In animals that received repeated exposure to shock over 14 consecutive days, MHPG concentrations remained elevated over a 24 hr period. Reexposure to the stressor further increased utilization of NE, but in contrast to the acute stress group, NE concentrations were also elevated. Brief shock reexposure 14 days after an acute stress session induced a marked decline of NE levels, whereas the same treatment applied to chronically stressed animals resulted in increased NE and MHPG concentrations. Thus it appears that the mechanisms responsible for the NE alterations engendered by both acute and chronic stressor application are subject to sensitization (or conditioning) processes. Whereas acute stress increases vulnerability to NE reduction, chronic stress predisposes the organism to increased concentrations upon subsequent aversive stimulation.

339.3 ROLE OF FOREBRAIN NOREPINEPHRINE (NE) IN STRESS-INDUCED DEFICITS IN CHOICE PERFORMANCE. T.R. Minor*, M.A. Pelleymounter*, and S.F. Maier. Dept. of Psych., Univ. of Colorado, Boulder, CO 80303.

We have shown previously that exposure to inescapable, but not escapable electric shock impairs later choice-escape learning. The deficit occurs only when a salient task-irrelevant cue is present during choice testing and is characterized by an inability to ignore the irrelevant cue. Experiments reported here examined the possibility that this effect results from NE depletion in the ascending dorsal tegmental bundle (ADTB) during exposure to inescapable shock.

Rats were exposed to 0, 40, 80, or 120 inescapable tail shocks in an attempt to produce graded NE depletion among groups. Twenty-four hr later, rats from each shock condition received 5 or 100 choice-escape trials in the presence of an irrelevant, intramaze light cue. Monoamines (NE, DA, 5-HT) in hippocampus and telencephalon were measured for rats receiving 5 choice trials. Of interest was whether group differences in NE in these projection areas of the ADTB emerged early in training, and if so, were they predictive of overall choice performance in each shock condition.

DA and 5-HT did not differ among groups. After 5 choice trials, telencephalon NE was comparable in 0- and 40-shock groups, but was reduced by 20 and 27%, respectively, in 80- and 120-shock groups. Depletion of hippocampal NE occurred only in the 120-shock condition. Choice performance paralleled these changes in NE. Whereas the 40-shock group performed as well as no-shock controls, moderate and severe impairment occurred, respectively, in the 80- and 120-shock conditions.

Subsequent studies have extended these observations. Bilateral 6-hydroxydopamine lesions of the ADTB mimicked the effects of inescapable shock. Performance of lesioned rats was impaired only when an irrelevant cue was present on choice trials. Vehicle controls were unimpaired regardless of cue condition. Further, the choice deficit can be prevented by bilateral microinfusion of chlordiazepoxide into the region of n. locus coeruleus, the origin of the ADTB, just before exposure to inescapable shock.

These data are consistent with the notion that the ADTB is involved in attention-like processes and suggest that deficits in stimulus selection following exposure to inescapable shock may arise from stress-induced depletion of forebrain NE.

339.4 NEONATAL FOREBRAIN NOREPINEPHRINE LOSS ELIMINATES REARING EFFECTS IN THE RAT. B.A. Pappas, M. Saari*, J. Smythe*, L. O'Shea*, S. Murtha*, K. Stange* and R. Ings*, Psychol. Dept., Carleton Univ., Ottawa, Ont., K1S 5B6 and Nipissing Univ. College, North Bay, Ont.

The effects of restricted visual experience during "critical periods" require intact cortical NE (Kasamatsu et al., J. Neurophysiol., 1981). We have also recently shown that neonatal 6-OHDA depletion of forebrain NE in rats eliminates enriched rearing effects on behavior and several measures of regional brain weight and catecholamine levels (O'Shea et al., Europ. J. Pharmacol., 1983). We further examined this by detailed behavioral testing and HPLC assay of regional brain monoamines and metabolites.

Newborn male rats were administered sc 6-OHDA or vehicle (VEH), reared from 25 to 60 days in isolate (IR) or enriched -social (ER) conditions and were then tested in either the Lashley Type III or Hebb Williams mazes. The latter group was sacrificed for HPLC assay. Behavioral testing and assays were performed blind to treatments. The assays are as yet incomplete and will be reported at the meeting.

ER improved Lashley maze performance of the VEH but not the 6-OHDA rats. In fact, performance of both the ER and IR 6-OHDA groups was equivalent to that of the ER VEH rats and all of these groups showed performance superior to that of the IR VEH rats. Similarly, for the Hebb Williams maze, the VEH ER animals made fewer errors than did the VEH isolates. No differences were observed between the ER and IR 6-OHDA rats. However, both 6-OHDA groups solved the problems as efficiently as did the ER VEH rats and more efficiently than did the VEH isolates. Thus, ER enhanced problem solving ability in normal rats but failed to do so in neonatal NE depleted rats. Significantly, however, both enriched and isolated NE depleted rats showed problem solving ability which was as good as that of the VEH enriched rats.

We conclude that NE depletion eliminates the effects of isolated rather than enriched rearing. Second, the interpretation of enriched rearing experiments should focus not on the behavioral/morphological effects of enrichment (which approximates the normal habitat of the rat) but on the effects of impoverishment (which, in the animal's normal habitat would be an aberrant rearing state). Finally, the results support the hypothesis that forebrain NE is essential to the consequences of restricted environments as suggested by Kasamatsu et al.

339.5 THE EFFECT OF NOREPINEPHRINE DEPLETION BY XYLAMINE ON INVESTIGATORY BEHAVIOR AND ON BRAIN WEIGHTS WITH ENRICHED REARING. S. Benloucif, M.R. Rosenzweig and E.L. Bennett Dept. of Psychology and Lawrence Berkeley Lab., University of California, Berkeley, CA 94720.

Current research has implicated the catecholamine neurotransmitter norepinephrine (NE) in investigatory behavior (Flicker & Geyer, 1982), arousal, and learning and memory (Kety, 1970, 1972), and neural plasticity (Kasamatsu et al., 1981). The view that NE is involved in learning and neural plasticity is supported by recent reports that depletion of brain NE by 6-hydroxydopamine (6-OHDA) reduces the brain weight increases normally induced by enriched rearing (Mirmiran et al., 1983; O'Shea et al., 1983). This experiment examined the role of arousal and investigatory behavior in mediating this NE effect on brain weights and learning. Rats were administered the permanent NE depletor xylamine (N-2-chloroethyl-N-ethyl-2-methylbenzylamine) or saline at weaning and assigned to either enriched (EC) or standard housing conditions. Behavior was observed in both a novel investigation arena and the home cage. Xylamine (XYL) treated and control rats from the standard condition were tested for acquisition and retention measured by a spatial learning task. Brains of the XYL treated and control rats from EC were weighed in order to determine whether NE depletion could counteract the brain weight changes that are normally induced by enriched rearing. Results revealed that XYL treatment decreased both investigatory behavior in the novel arena and also tended to decrease home cage activity. Brain weights for XYL-treated rats in EC were also reduced relative to those of control rats in EC. Acquisition of the learning task was unaffected by NE depletion. It is suggested that the decrease in brain weights found by this laboratory and others with NE depletion is due in part to a secondary effect of a decrease in investigatory behavior, rather than solely to a primary effect of NE on neural growth.

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339.6 LOCUS COERULEUS UNIT ACTIVITY IN CAT: BEHAVIORAL AND STATE CORRELATES. B. L. Jacobs, K. Rasmussen and D. Morilak. Neurosci. Prog., Princeton Univ., Princeton, NJ.

Recently, several studies have examined the activity of locus coeruleus (LC) neurons in behaving animals. We report here on a series of experiments that continues this line of investigation in the cat. The present study focusses on the behavioral and state correlates of LC neuronal activity, with a particular emphasis on affect, arousal, and movement. The following two abstracts deal with the effect of anxiolytic and anxiogenic agents, and morphine, respectively. Single unit activity was recorded by means of movable 32 and 64 μm dia, insulated, nichrome wires implanted in two bundles of six each above the LC (P 4.0 L 3.0 H -1.9). Neurons in the area of the LC were initially identified as noradrenergic by their long duration action potentials, slow and somewhat regular discharge pattern, excitation - inhibition response to paw or tail pinch, and complete cessation of activity during REM sleep. This identity was confirmed further by the complete suppression of neuronal activity following a systemic injection of a low dose of the α_2 agonist clonidine (25 μg/kg i.p.). Finally, all neurons displaying such characteristics were histologically localized within the LC (this characteristic activity was not encountered on penetrations outside of the LC). During an undisturbed quiet waking state, LC neurons discharge at a slow rate ($\bar{X}$=0.9 spikes/sec) with a somewhat regular pattern. This activity can be driven by simple sensory events (click or flash) with latencies of ∿40-50 msec and durations of ∿100-200 msec. Typically, under these conditions, one spike is elicited by each stimulus presentation. The magnitude of this sensory-evoked unit response can be modulated by conditions that either distract the animal's attention away from these stimuli (e.g. decreased by rats in the experimental chamber) or under conditions in which the animals focus upon the stimuli (e.g. increased by conditioning trials). General activation of the cat or increased motor activity, for example, eating, drinking, or running on a treadmill, produces at most a modest increase in discharge rate. However, when the activation has an added affective component, for example, air puff to the face or conditioned emotional response (CER) training, there is often a dramatic increase in LC unit activity (phasically, up to 15-fold, tonically, up to 5-fold). In support of previous investigators, these data and those presented in the following abstract indicate that LC unit activity is positively correlated with affect and/or autonomic activation. (Supported by NIMH grant MH 23433).

339.7 LOCUS COERULEUS UNIT ACTIVITY IN BEHAVING CATS: EFFECT OF ANXIOLYTIC AND ANXIOGENIC DRUGS. K. Rasmussen, D. Morilak, and B.L. Jacobs, Prog. Neurosci., Princeton Univ., Princeton, NJ.

Noradrenergic neurons in the area of the locus coeruleus (LC) have been hypothesized to play a critical role in anxiety. This has been partly supported by studies showing: 1) anxiogenic drugs, e.g. yohimbine, increase LC unit activity in chloral hydrate (CH) anesthetized rats; and 2) anxiolytic drugs, e.g. diazepam, decrease LC unit activity in CH anesthetized rats. In an extension of these studies, we have examined the effects on LC unit activity of yohimbine and diazepam in freely moving cats. The general methodology and the criteria for identifying neurons as NE, as well as their response to sensory stimuli are described in the preceding abstract. Spontaneous unit activity and unit response to sensory stimuli (click or flash) were examined before and after drug administration. Yohimbine hydrochloride (2 mg/kg i.p.), a drug which is presumed to be anxiogenic in both animals and man, led to behavioral activation of the cat and produced an increase in spontaneous LC unit activity (∿80%). In addition, yohimbine produced a relatively larger increase in the LC unit response to sensory stimuli (∿200%). Similar results on LC unit activity (i.e. increased spontaneous activity and relatively larger increased sensory response) were obtained after conditioned emotional response (CER) training (a condition which is presumably anxiety-inducing). Diazepam, a benzodiazapine anxiolytic, (0.1 - 2 mg/kg i.p.) did not produce a significant decrease in the spontaneous activity of LC neurons as would have been expected from studies of CH anesthetized rats. However, at a dose that produced no behavioral signs of sedation or ataxia, diazepam (0.25 mg/kg i.p.) greatly decreased (∿ 90%) the sensory evoked unit response of LC neurons. These data may explain how diazepam can be anxiolytic without being sedating i.e. it does not affect the spontaneous activity of LC neurons but it does dramatically decrease the responsiveness of these neurons to environmental stimuli. Reciprocally, anxiogenic agents may act in part by producing a relatively larger effect on sensory evoked unit activity of LC neurons as compared to their effect on spontaneous LC unit activity. These data support the general hypothesis that the LC plays an important role in affect and/or autonomic activation. In preliminary experiments on serotonergic neurons in the dorsal raphe nucleus, we have also observed preferential effects upon sensory evoked unit responses under these experimental conditions. (Supported by NIMH grant MH 23433).

339.8 LOCUS COERULEUS UNIT ACTIVITY IN BEHAVING CATS: SYSTEMIC MORPHINE HAS NO EFFECT. D. Morilak, K. Rasmussen, and B.L. Jacobs. Prog. Neurosci., Princeton Univ., Princeton, NJ.

Noradrenergic (NE) neurons in the area of the locus coeruleus (LC) have been implicated in several aspects of the action of opiates. This is consistent with: the dense aggregation of opiate binding sites in the LC of the rat; the decrease in LC unit activity produced by morphine administered to anesthetized rats; and the finding that the α_2 agonist clonidine alleviates the symptoms of opiate withdrawal in several species. In the course of our studies on LC single unit activity in freely moving cats, we examined the effects of systemic administration of morphine. The general methodology and the criteria for identifying neurons as NE are described in a preceding abstract (B.L. Jacobs et al.). After a baseline recording period, during which LC unit activity was sampled in a variety of behaviors and states, cats were administered morphine sulfate (0.5, 2.0 or 4.0 mg/kg i.p.) and unit activity was continually examined for the next hour and then periodically sampled for the next two hours. None of the doses of morphine produced a significant decrease in LC unit activity from the baseline level ($\bar{X}$ = 0.9 spikes/sec; total N=12). In fact, over half of the cells showed a significant increase in activity (Md=48%). The two highest doses produced clearcut signs of analgesia as measured by several different tests (e.g. tail flick and formalin test). In order to try to reconcile our results with the previous reports that morphine significantly decreased the activity of LC neurons in chloral hydrate (CH) anesthetized rats (e.g. Korf et al., 1974), we conducted similar studies in the cat. Somewhat surprisingly, CH anesthesia alone (200-300 mg/kg i.p.) produced a significant increase (2-4 fold) in LC unit activity. When morphine (2.0 mg/kg) was then administered, it produced a significant decrease in LC unit activity, but one which did not go below the pre-anesthetic baseline. Thus, morphine's depression of LC unit activity in rats may be at least partially attributable to an interaction with CH anesthesia (see Brain Res. (1984) 291:63-72). However, these discrepant data may also be accounted for by important species differences. In cat, NE neurons in LC are much more spatially dispersed than in the rat, where they form a compact homogeneous nucleus. This dispersion may alter the response of cat LC neurons to morphine and/or CH. It is also well known that cats may manifest a mania response to high doses of morphine whereas rats are exclusively sedated by various doses of morphine. (Supported by N.I.M.H. grant MH 23433).

339.9 NEUROCHEMICAL CORRELATES OF DOMINANCE BEHAVIOR IN THE SQUIRREL MONKEY. K. A. Greene*, C. L. Coe*, K. F. Faull, R. J. King, Jr., J. D. Barchas, and S. Levine. Dept. of Psychiatry, Stanford University, Stanford, CA 94305.

Monoamine metabolites were measured in male squirrel monkey cisternal CSF in an effort to examine the effect of dominance behavior on CNS neurotransmitter turnover.

Pairs of animals were evaluated in three conditions: stable pairs, recently formed pairs, and pair severance. The stable condition consisted of animals paired for at least 4 weeks prior to experimental manipulation and provided stable dominance relationships as a control. In the recently formed pair condition, animals were individually housed for at least 4 weeks and were then paired at the initiation of the experiment. In the last condition, animals initially paired for at least 4 weeks prior to experimentation were separated from their respective paired animal and housed individually at the initiation of the experiment. Following ketamine administration, CSF samples were obtained from the cisterna of all animals during the last week of the 4-week conditioning period for baseline measurements and, eight days later, at one day post manipulation. At the same or similar times, paired animals were assessed for dominance using the water test, food test, or sex test.

Measurements of the concentrations of the metabolites DOPAC and HVA (derived from dopamine), MHPG (derived from norepinephrine and epinephrine) and 5-HIAA (derived from serotonin) were made using GC/MS. The only significant findings that emerged involved MHPG; a significant association between subordinant status and high levels of MHPG ($n=9$, $p<0.02$, Wilcoxon paired difference test) and a significant interaction between housing conditions (paired vs. isolated) and social status (dominant vs. subordinant) with respect to concentrations of MHPG ($F(1,16)=6.17$, $p<0.025$). Furthermore, the suggestion that dominance is an important variable in determining CSF MHPG concentrations is supported by the fact that in the third condition, in which the animals were initially paired and then isolated, mean values of MHPG increased in dominant males upon isolation while the same parameter decreased in the subordinant males when they were isolated from their dominant counterparts; after pair severance CSF MHPG no longer reflected the former dominance relationships.

These data provide evidence for an association between dominance behavior and central noradrenergic activity in a nonhuman primate species.

339.10 YOHIMBINE-PRECIPITATED ABSTINENCE SYNDROME FOLLOWING CONTINUOUS INFUSION OF CLONIDINE. D. H. Malin, R. J. Exley*, R. F. Hamilton*, and R. J. Townsend*. Univ. of Houston-Clear Lake, Houston, TX 77058.

The alpha-2 adrenergic agonist clonidine has a number of opiate-like actions. Might chronic suppression of noradrenergic activity by clonidine produce a dependence-like state? A previous study from our laboratory demonstrated many opiate-abstinence-like behaviors in rats one day after termination of 5 days of continuous clonidine infusion.

The alpha-2 antagonist yohimbine reverses the effects of clonidine. The present experiment determined whether yohimbine injections in clonidine infused rats could precipitate an immediate withdrawal syndrome, analogous to naloxone after morphine exposure.

Ten female rats were habituated to an O2 consumption chamber and their baseline consumption was determined. They were then subcutaneously implanted with Alzet 2001 minipumps under ether anethesia. Five rats received pumps filled with saline alone, while five were infused continuously with 0.033 mg/kg/hr clonidine. After 120 hours, each rat was observed 15 min. for standard behavioral signs seen in opiate withdrawal and its O2 consumption retested. Each rat was then challenged with 3 mg/kg yohimbine i.p. and retested 10 min. later for behaviors and O2 consumed. As Table 1 shows, both groups initially had few behavioral signs and near-baseline O2 rates. Yohimbine, however, produced significant increases in both behavioral abstinence signs (particularly abdominal writhes) and O2 consumption in the clonidine infused group. Additionally some seizures were noted.

Table 1	Abstinent-Like Signs		O2 as % of Baseline	
	Saline	Clonidine	Saline	Clonidine
Pre-Yohimbine	3.0 ± 1.4	1.2 ± 0.58	$105\pm3.88\%$	$95\pm9.7\%$
Post-Yohimbine	2.6 ± 0.87	13.6 ± 1.43*	$98\pm7.05\%$	$136\pm9.4\%$*

*Significantly different from saline controls.

A second experiment employed similar procedures except for a clonidine infusion rate of 0.01 mg/kg/hr. Yohimbine injection resulted in significant numbers of abstinence signs and clonic seizures in clonidine infused rats. Sudden release from chronic suppression of noradrenergic activity appears to produce certain effects ordinarily associated with opiate or alcohol abstinence syndrome.

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339.11 THE EFFECTS OF ALPHA$_2$-ANTAGONISTS ON AMPHETAMINE-INDUCED INCREASES IN LOCOMOTION AND STEREOTYPY. D. Luttinger and M.E. Durivage.* Department of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, NY 12144

Alpha$_2$ adrenergic antagonists have been extensively utilized as a pharmacological tool to study the effects mediated via alpha$_2$-adrenergic receptors. We were interested in studying the behavioral effects of alpha$_2$ antagonists. This study was designed to investigate the interactions of alpha$_2$ antagonists on amphetamine-induced behaviors.

Male Swiss-Webster mice (Taconic Farms) weighing 14-35 gms group housed with food and water ad libitum were used. Both locomotor activity (distance travelled) and stereotypies (number) were measured with Columbus Instrument Opto-Varimex cages (41.4 x 42.3 cm). The alpha$_2$-antagonists were injected s.c. 15 minutes before an i.p. injection of 3 mg/kg d-amphetamine. The mice were then placed in the activity cages and allowed 25 minutes to habituate at which point locomotor activity and stereotypies were assessed during the subsequent 10 minutes.

Amphetamine, when administered alone, routinely increased both the distance travelled and the number of stereotypies. The alpha$_2$-adrenergic antagonists RX781094, rauwolscine, RS 21361, tolazoline and yohimbine attenuated the amphetamine effect on distance travelled in a dose-related manner. The minimally effective dose of RX781094 and rauwolscine was $\leq$ 1 mg/kg and for yohimbine was $\leq$ 3 mg/kg. Tolazoline was active at 10 mg/kg. RS 21361 was also active; however, at a higher dose (100 mg/kg). Thus, the relative potencies for the alpha$_2$ antagonists were comparable to those reported in other tests. The antagonists attenuated the amphetamine induced increases in locomotor activity often at doses which did not affect locomotor activity when given alone. Interestingly amphetamine induced stereotypies were only affected at higher doses of the antagonist which also inhibited the number of stereotypies when given alone. Clonidine, an alpha$_2$ adrenergic agonist, which alone decreases locomotor activity and stereotypy did not affect amphetamine induced increases in behavior. Corynanthine, an isomer of yohimbine which is relatively selective for alpha$_1$ receptors, was inactive in this procedure. Thus further suggesting the effect observed with the compounds is due to alpha-2 adrenergic antagonism.

These effects were qualitatively similar to those observed with the atypical antipsychotic agent, clozapine. Typical antipsychotic agents (e.g., haloperidol) routinely inhibit amphetamine actions on both locomotor activity and stereotypy at comparable doses. The present data with the alpha$_2$ adrenegic antagonists suggest that they may represent a novel class of antipsychotic compounds with a reduced risk of extrapyramidal side effects.

339.12 ENDURING CHANGES IN BEHAVIOR PRODUCED BY AMPHETAMINE. Terry E. Robinson. Department of Psychology & Neuroscience Laboratory Building, The University of Michigan, Ann Arbor, MI 48104-1687.

Humans who are frequently exposed to psychomotor stimulant drugs sometimes develop a psychotic-like state that is nearly indistinguishable clinically from paranoid schizophrenia. Partly for this reason there has been a great deal of interest in the neurobehavioral effects of drugs like amphetamine (AMPH), and particularly in the long-lasting effects of repeated exposure to AMPH. There are many studies showing that some of the motor stimulant effects of AMPH are progressively enhanced with repeated administration. However, the development of enduring changes in brain and behavior produced by AMPH may be influenced by a variety of variables that are not well understood. In the experiments reported here we examined the influence of single vs. multiple injections, inter-test interval, sex, gonadal hormones and conditioning on the development of behavioral sensitization ('reverse tolerance'), by quantifying rotational behavior in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra. The results indicate that: (1) a single injection of a low dose of AMPH enhances the rotational behavior induced by a second injection of AMPH given up to 12 weeks later; (2) multiple, weekly injections of AMPH produce a progressive enhancement in rotational behavior, over-and-above that produced by a single injection; (3) under some conditions greater sensitization is produced by weekly than by daily AMPH injections, suggesting a kindling-like phenomenon; (4) female rats show a more robust sensitization than males following single or multiple injections of AMPH; (5) this sex difference may be due to the suppression of sensitization by an androgen, because removal of testicular hormones potentiates sensitization; and (6) the long-lasting sensitization of rotational behavior produced by infrequent injections of AMPH can not be explained by drug-environment conditioning effects, but is probably due to a persistent AMPH-induced change(s) in brain catecholamine systems. The results illustrate an intriguing example of neuroplasticity that may have considerable clinical relevance.

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339.13 AMPHETAMINE CONDITIONED TASTE AVERSION PRODUCED BY MICROINJECTION INTO AREA POSTREMA REGION. G.D. Carr and N.M. White, Dept. of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal, Quebec, Canada H3A 1B1

Amphetamine is known primarily for its rewarding properties, but a contrasting effect is the tendency of animals to show an aversion towards tastes that have previously been paired with the drug. This effect is known as a conditioned taste aversion (CTA) and can also be produced by noxious treatments (eg. lithium) and by other rewarding drugs (eg. morphine). While the CTA to toxins can be blocked by lesions of area postrema, these lesions do not affect the CTA to amphetamine (Berger, et al., J. comp. physiol. Psychol., 82: 475-479, 1973). One clue to the substrate of the effect is that the amphetamine CTA can be attenuated by selective lesions of brain dopamine (Wagner, et al., Pharmacol. Biochem. Beh., 14: 85-88, 1981). We therefore examined the ability of amphetamine microinjections into several brain sites containing dopamine to produce a CTA. Rats with surgically implanted cannulae aimed at the nucleus accumbens, caudate nucleus, amygdala, or area postrema region drank a maple sucrose solution for 15 minutes and were immediately microinjected with 10 or 20 ug d-amphetamine (in 0.5 uL) or 0.5 uL saline. The next day (test) they were offered the maple-sucrose solution in the absence of the drug. Injection of amphetamine into the accumbens, caudate or amygdala produced no significant change in test day consumption relative to the saline controls. However the amphetamine injected into the region of area postrema caused a significant ($t(12)=3.48$, $p<.005$) decrease in consumption of the paired flavour relative to saline-injected controls. A slightly stronger aversion was produced by using three flavour-drug pairings ($t(12)=4.82$, $p<.001$). The CTA did not occur in a group in which the cannulae were aimed 1mm antero-ventrally to the effective site. We interpret the results as suggesting a role for the area postrema region in mediating amphtamine-induced CTA. This is consistent with findings of endogenous dopamine in the area postrema and surrounding structures. The fact that lesions of the area postrema itself do not block amphetamine CTA indicates that the structure is not critical for the effect. Combined with the present findings, it suggests that structures in the region around the area postrema such as the nucleus of the solitary tract are involved in the effect.

339.14 DISSOCIATION OF SCOPOLAMINE AND D-AMPHETAMINE-INDUCED HYPERACTIVITY. R.E. Davis, J.P. Symons, S. Yoder and J.G. Marriott. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI. 48105.

Catecholamine agonists such as, d-amphetamine, and the anticholinergic, scopolamine, elicit excessive and stereotyped motor activity. This drug-induced activity generally has been studied in large open fields. It is difficult to distinguish between these two types of drug-induced hyperactivity in this environment without detailed and prolonged behavioral analyses. We have developed a simple and rapid method to reliably distinguish between motor activity increases induced by catecholamine agonists and anticholinergics. This method is based on differences in open field locomotor activity and swimming activity patterns elicited by these agents.

Male, hooded, Long-Evans rats were administered intraperitoneally, either the catecholamine agonist, d-amphetamine (0.32, 1.0 3.2 mg/kg) or the anticholinergic, scopolamine (0.1, 0.32, 1.0 mg/kg). Thirty minutes later these animals were placed in a white plastic enclosure (133 x 133 x 67 cm). For swimming activity tests the enclosure was filled to a depth of 33 cm with cold water (22 C). For open field activity tests the enclosure was empty. Activity was monitored continuously for the next 5 mins using a computerized videotracking system. The total distance traveled and the pattern of activity was recorded for each animal and served as the dependent measure.

In the open field d-amphetamine increased the total distance traveled while scopolamine at these doses did not. Conversely, scopolamine dramatically increased total swimming distance while d-amphetamine did not alter this form of activity. In addition, the pattern of swimming activity elicited by scopolamine was extremely stereotyped and thigmotaxic.

These data demonstrate clearly that swimming and open field locomotor activity are differentially sensitive to scopolamine and d-amphetamine. This differential sensitivity provides a means for rapidly distinguishing these forms of drug elicited hyperactivity and suggests that swimming activity and open field locomotion are controlled by different neurochemical systems.

339.15 CHOLINERGIC CONTROL OF SOCIAL PLAY. J. Panksepp, T.L. Sahley* and L.N. Normansell*. Dept. of Psych., Bowling Green State University, Bowling Green, OH 43403

Social play (as assayed in rats) is highly sensitive to diverse environmental and pharmacological manipulations. Since many drugs can reduce play, criteria are needed to sift specific controls from other disruptive influences. Specific functions are implicated when agonists and antagonists have opposite effects as well as when they cancel each others effects. By such a criterion, only brain opioids presently have adequate support for specific modulatory control of play, for opiate agonists increase and antagonists decrease play. We now report similar trends for the nicotinic cholinergic system.

Social play of Long-Evans rats was studied by measurement of pinning behavior during paired-encounters following treatment with various cholinergic drugs. Nicotine (NIC) (.125-.5 mg/kg) systematically reduced play (down 25-80%), while the same low doses of mecamylamine (MEC) modestly increased play (up 20 to 70% depending on conditions). Doses of MEC above 1.5 mg/kg reduced play. Effects of MEC were more apparent following mild social deprivation than in chronically isolated animals. The almost total suppression of play by 0.5 mg/kg of NIC could be reversed completely by 0.5 mg/kg MEC but not by scopolamine, which by itself reduced play. When one animal of a play pair was treated with 0.5 mg/kg of NIC, it tended to become submissive; MEC had no clear effect on play dominance.

These results suggest specific modulation of play by nicotinic cholinergic synapses. It is noteworthy that another social behavior, separation induced distress vocalization, is also modulated by the same system (Sahley, Eur. J. Pharmacol., 1981, 72, 261), suggesting possible convergent influences of these systems on social affect. However, while opiates decrease crying and increase play, nicotine decreases both, and MEC increases play without affecting crying, while naloxone decreases play and increases the tendency to cry.

339.16 DOPAMINERGIC SUBSTRATES OF PLAY. J. Cox*, L. Schoen*, L. Normansell*, J. Rossi III, S. Siviy, and J. Panksepp. Dept. of Psych., Bowling Green State University, Bowling Green, OH 43403

Previous work has established that both stimulants and neuroleptics decrease rat play, as measured by pinning in a paired-encounter procedure, but the roles of dopamine(DA) and norepinephrine(NE) in these effects need to be clarified. The dose response characteristics of d- and l-amphetamine were quite similar, suggesting that the reduction in play produced by stimulants may be mediated largely by NE in the brain. Haloperiodol(0.1 mg/kg), tested in interaction with the lowest effective dose of l-amphetamine (0.25 mg/kg) did not reverse play inhibition. Clonidine was found to be highly effective in reducing play (at doses above 1 ug/kg) and this effect could be partially reversed by the alpha-2 antagonist yohimbine (0.5 mg/kg).

Other evidence suggests that DA activity facilitates play. Low doses of d-amphetamine, but not l-amphetamine (0.25 mg/kg) gradually promote play dominance. Conversely, a low dose of haloperidol (0.1mg/kg) given to one animal of each play pair reduces that animal's dominance. Likewise, when dominant rats of play pairs receive haloperidol (.05 or .1 mg/kg), the established dominance relationships collapse. Further, a slight increase in play is obtained when both partners receive apomorphine (0.5 mg/kg). Likewise, preliminary data show that bilateral 6-OHDA lesions of the ventral tegmental area of pre-weanling rats reduces subsequent play substantially, however, this may be due to concurrent weight reduction.

These results implicate brain DA circuitry in the facilitation of social play. Since social play is a rewarding activity, as indicated by the willingness of animals to negotiate mazes for opportunities to play, these results suggest that certain aspects of social reward are elaborated by dopaminergic substrates in the brain.

339.17 CENTRAL CHOLINE INJECTIONS REVERSE BEHAVIORS INDUCED BY HIPPOCAMPAL DAMAGE. J. E. Springer, J. P. Ryan, J. Johnston,* and R. L. Isaacson. Department of Psychology and Center for Neurobehavioral Sciences, SUNY-Binghamton, Binghamton, NY 13901.

We previously reported that systemic choline treatment reverses some of the open field behaviors seen following hippocampal damage (Springer and Isaacson, (1983) Neurosci. Abs. 9, 554). It was suggested that the effects of choline might be centrally mediated since pretreatment (icv) with hemicholinium-3 (a purported blocker of choline uptake) blocked the behavioral efficacy of choline. Therefore, we initiated experiments to further characterize the possible central effects of choline in animals with hippocampal damage.

Rats received either sham, cortical, or hippocampal lesions by aspiration. Also, each animal was implanted unilaterally with a stainless steel cannula aimed at the ventricular region. Starting at 6 or 27 days after surgery, the animals were observed for 4 consecutive days in an open field. Each animal received 1.0 µl of saline vehicle, or 0.01, 0.1, or 1.0 µg of choline chloride injected through the ventricular cannula. Ten min. later the animals were observed in the open field for a period of 10 mins.

At the early post-surgery test session, choline at the 0.1 and 1.0 µg doses reversed the high levels of locomotor activity normally seen following hippocampal damage. In these brain damaged animals, these two doses of choline also increased the amount of time the animals spent performing each rear and hole-poke. These times are usually substantially reduced after hippocampal ablation. At the later post-surgery test session choline was also effective, but only at the 1.0 µg dose.

It is suggested that some of the behaviors seen following ablation of the hippocampal formation may be attributable to secondary changes in some central cholinergic system.

340.1 IDENTIFICATION OF AN IMMUNOREACTIVE ANALOGUE OF ERYTHROCYTE SKELETAL PROTEIN 4.1 IN PIG BRAIN. S.R. Goodman*, I.S. Zagon, L.A. Casoria*, and D.B. Coleman* (SPON: T.A. Lloyd). M.S. Hershey Med. Ctr., Pennsylvania State Univ. Hershey, PA 17033

RBC protein 4.1 is an essential component of the erythrocyte membrane skeleton, which strengthens the interaction between spectrin and actin by forming a spectrin-4.1-actin ternary complex. The erythrocyte membrane skeleton is responsible for controlling red cell shape, elasticity, and membrane structural stability. Brain contains an immunoreactive analogue of spectrin, which is an $\sim 1 \times 10^6$ M_r, $(\alpha\beta)_2$ tetramer (α=240 K Dal, β=235 K Dal), and can crosslink actin filaments bivalently by binding end-on to the f-actin. In this study, immunoautoradiography utilizing a monospecific anti-pig rbc protein 4.1 antibody has demonstrated staining of an 87K Dal 4.1 immunoreactive analogue in pig brain membranes. Two dimensional tryptic peptide analysis of protein 4.1 immunoprecipitated from triton X-100 solubilized pig rbc and brain membranes, demonstrated a 50% overlap between rbc and brain protein 4.1. We have localized brain protein 4.1 within pig cerebellum by indirect immunofluorescent observation utilizing the anti-pig rbc 4.1 antibody. Low magnification observation of stained 10 µm sections indicated bright staining of granule cells in the internal granule layer, with low level staining of the molecular and medullary layers. Higher magnification observation revealed bright staining of the cortical cytoplasm of internal granule neurons and Purkinje neurons, with no observable staining of their nuclei. In the medullary layer although there was no staining of myelin, there did appear to be faint staining of the cortical cytoplasm of glial cells. The staining pattern observed with anti-rbc 4.1 IgG and anti-rbc spectrin IgG appeared to be identical in the pig cerebellum. As brain protein 4.1 is immunologically and structurally related to rbc protein 4.1, and because of its co-localization with brain spectrin in neural cells, brain protein 4.1 may play a similar functional role to its rbc analogue.

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340.2 ULTRASTRUCTURAL STUDIES OF MEMBRANE SPECIALIZATIONS IN THE GOLDFISH PREOPTIC AREA. W.A. Gregory, D.H. Hall, and M.V.L. Bennett, Dept. Neuroscience, Albert Einstein Coll. Med., Bronx, NY 10461.

Preoptic neurosecretory cells of fish, and homologous mammalian cells, are unusual in having large areas of direct soma to soma apposition without intervening glia. The amount of apposition can be affected by physiological state in rats (e.g. lactation, Hatton & Tweedle, Brain Res. Bull., 8, 197, 1982). In rats during lactation, oxytocin-containing neurosecretory cells fire synchronously, although the degree of synchronization has not been determined. Electrotonic coupling via gap junctions mediates synchronous firing in many instances, but it has also been proposed that direct appositions without gap junctions could serve the same function. In part to address this problem, the fine structure of the appositional regions was studied in goldfish. When brains were fixed with 4.0% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2), osmicated, en bloc stained in uranyl acetate, and embedded in Epon, definitive gap junctions were never seen between neurons, although they were common between glia. The addition of ruthenium red to the glutaraldehyde gave rise to occasional zones of close apposition between neurons. These structures had a pentalaminar appearance, while glial gap junctions were heptalaminar as in glutaraldehyde fixed material. Because of their pentalaminar appearance and absence in conventionally fixed material, these close appositions are probably artifacts. Cytoplasmic bridges were occasionally seen between neurosecretory cells in conventionally fixed material, as well as in ruthenium red plus glutaraldehyde and osmium perfused material. The bridges, which often appear artifactual, are a potential morphological substrate for the postfixational migration of HRP or fluorescent dyes that bind to proteins, and could account for the reported HRP coupling between these neurons (Reaves, Cumming & Hayward, Neuroscience, 7, 1545, 1982). In freeze fracture analysis, 100-200 nm diameter gap junctions are frequent. Since they are large enough to be visible in thin sections, they are probably located between glia. Thus, if the neurosecretory cells are coupled by gap junctions, the junctions must be too small to be visible in thin section, and cannot involve large aggregates of particles. Gap junctions of few particles cannot be excluded.

340.3 A POSSIBLE ROLE FOR THE PLASMA MEMBRANE IN THE REGULATION OF γ-GLUTAMYL TRANSPEPTIDASE. J.L. Flagg-Newton* and L.E. DeBault University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, 73190.

Rat C6 glioma cells were maintained in culture in a series of growth media which varied fundamentally in terms of their serum concentration and primary carbon source. The effect of these parameters on γ-glutamyl transpeptidase (γGTP), a membrane bound enzyme, was investigated in order to establish their role in its phentotypic expression. The specific response of the cells to growth media were found to be passage dependent. For high, but not low passage cells, growth in medium containing 10% fetal bovine serum (FBS) plus glucose as the primary carbon source resulted in substantial increases in γGTP. Only in the presence of 1% FBS plus galactose did both high, as well as low passage cells, exhibit a significant enhancement of γGTP. Irrespective of the source of carbon or cell passage, maximal rises in γGTP were consistently observed during peroids of little or no cell growth. Concomitant with the media related enhancement of γGTP was a substantial enhancement of glutathione, both reduced as well as oxidized species. The apparent relationship between γGTP and glutathione, the cells' dynamic thiol redox system, was further investigated by exposure of cells to agents known to modulate, directly or indirectly, intracellular levels of the thiol. Exposure to tertiary butyl hydroperoxide sodium selenite, and vinblastine sulfate as well as to isoproterenol or cyclic adenosine monophosphate (cAMP) resulted in moderate to marginal increases in γGTP. Only in one case, cell growth in glial conditioned medium, did γGTP induction occur independently of modulations in cellular glutathione. While these results show a strong correlation between γGTP induction and rises in cellular glutathione, caution must be exercised in suggesting a direct role for the thiol in the enhancement of γGTP activity in cells. Media factors which alter the cellular redox state, as indicated by fluctuations in glutathione, may in effect, alter the redox state of the plasma membrane. Thus, the altered membrane rather than glutathione itself may be the source of the observed media related modulations in γGTP.

340.4 INTRACELLULAR INVESTIGATION OF INJURY POTENTIALS AFTER NEURITE TRANSECTION NEAR THE CELL BODY IN CULTURE. J.H. Lucas*, G.W. Gross and J.B. Kirkpatrick† Dept. of Biology, Texas Woman's Univ., Denton, TX 76204 and Dept. of Pathology, Baylor College of Medicine, Houston, TX 75235.†

We have recently reported a multiple shot, low energy method of laser cell surgery at 337 nm for producing point lesions with high precision in monolayer cultures (G.W. Gross, J.H. Lucas and M.L. Higgins, J. Neurosci. 3:1979, 1983). During transection we have observed narrowing of the target area and disappearance of cytoskeletal elements. The ultrastructural changes are similar to those described in studies of mechanical trauma and are probably related to increases of free intracellular Ca^{+2}. Accordingly, we are applying this method to the study of transection trauma to single neurons by observing injury potentials (IP) during and after process amputation. We are also investigating the responses of injured cells to pharmacological interventions which may enhance membrane potential (MP) recovery and cell survival.

Neurites from mouse spinal neurons (3-5 weeks in culture) were amputated at distances of 10-200 um from the perikarya. The resulting changes in MP varied from total loss to near recovery. However, data from cells traumatized in normal culture medium have never shown complete recoveries during a 30 min. time span following surgery. The time course of the IP is a function of distance of the lesion from the cell body. In the vicinity of 30 um there is a surprisingly steep rise of the IP magnitude. Within this distance lesions produce greater than 85% MP losses. Beyond 30 um an initial depolarization peak is usually followed by a lower steady plateau. In this region the surprisingly steady IP never exceeds 50% MP loss and appears to decrease exponentially with the lesion distance.

Cytochalasin B added 0.5 h prior to surgery (10 ug/ml) has no obvious effect on the IP time course at any distance. However, the neurofilament protease inhibitor leupeptin (1mM) added 1 h before surgery has resulted in complete repolarization in several instances after 15 min. Although preliminary, these data suggest that the maintenance of cytoskeletal stability may play an important role in the recovery from this type of lesion. On the other hand, actomyosin-mediated contractile events do not appear to play a major role in either the injury or recovery processes.

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340.5 EFFECTS OF PHOSPHOLIPASE A_2 (PLA_2) AND OTHER ENZYMES ON BULLFROG SCIATIC NERVE. J.W. Kasckow, L.G. Abood, W.P. Hoss and R.M. Herndon, Ctr. Brain Res., Univ. Rochester Sch. Med. & Dent., Rochester, NY 14642.

The effects of purified PLA_2 from bee venom and other enzymes were investigated in an in vitro physiologic assay using frog sciatic nerve. Sciatic nerves were threaded through a plexiglass chamber which contained wells for stimulating, recording and ground electrodes along with a well in which the desheathed nerve could be exposed to various enzymes. Incubation with 1% trypsin in Ringers for 3 hr had no effect on conduction. Lower concentrations of chymotrypsin and protease also had no effect. Nerves were then treated with 1% trypsin for 3 hr and incubated with various glycosidases, PLA_2 or normal Ringers. Incubation with only Ringers caused a decrease in the compound action potential (CAP) height of only 9.2 ± 3% (mean ± standard deviation) from the original value as measured before addition of Ringers. Incubation with 1 mg/ml β-glucosidase caused a decrement of 5.6% and incubation with 2-3 mg/mlβ -galactosidase caused a decrement of 33%. Increasing concentrations of PLA_2 yield a typical dose response curve: PLA_2 concentrations of 0.2 mg/ml, 0.4 mg/ml and 0.8 mg/ml each caused the height of the CAP to decrease by 2.5 ± 3%, 29.5 ± 2% and 77.3 ± 1%, respectively, relative to its value before PLA_2 addition. The effects at the highest dose were irreversible; the decrement in CAP height caused by the 2 hr incubation could not be reversed by a further 1 hr wash with 2 mg/ml albumin. Incubation at 0.8 mg/ml PLA_2 along with 2 mg/ml albumin caused a decrement in CAP height of only 7.5%, a finding which suggests that the hydrolytic products are involved. We are now investigating the effects of the metabolic products of PLA_2. 10 mg/ml arachidonic acid caused a 63% drop in the height of the CAP; oleic acid had no effect at the same concentrations. At concentrations of L-α-lysophatidylcholine (mostly palmitic and stearic acids) in excess of 1 mg/ml, there occurred conduction block as well as extensive myelin damage. Morphologic analysis of PLA_2 effects demonstrate paranodal disruption at the light microscopic level. At the EM level, the paranodal damage is characterized by bubbling and vesiculation at the cytoplasmic loops and increased incidence of Schmidt-Lantermann clefts with intact axolemmas.

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340.6 PROTEIN- AND CALCIUM-CATALYZED AGGREGATION AND FUSION OF ISOLATED CHROMAFFIN GRANULES. Stephen J. Morris*, Neurotoxicology Section, NINCDS, NIH, Bethesda MD, 20205, USA

Since our first observations that chromaffin granules will aggregate and fuse in the presence of Ca^{2+}, we have been studying the morphology and kinetics of these events as a possible model for exocytosis. An extensive analysis showed that granules aggregate with near diffusion-controlled kinetics and that K^+ would aggregate the membranes to a limited extent (1). We report the development of a real-time assay for granule fusion and comment on the ability of proteins to act as catalysts for fusion events.

Chromaffin granules were labelled with small unilamellar vesicles (SUV) made from NBD and/or lissamine rhodamine sulfonyl phosphatidyl ethanolamine as donor and acceptor fluorophores. The mechanism seemed to be fusion of the SUV with the granule membrane rather than transfer of label.

Kinetics of granule-granule fusion can be followed either by resonance energy transfer from donor to acceptor fluorophores, or by donor quenching. Fusion rates are 5-10 fold slower than aggregation, demonstrating that aggregation is not rate-limiting and suggesting extensive membrane rearrangements are needed after contact. Fusion of freshly prepared granules can be initiated by mM concentrations of calcium; magnesium is less effective; Mg-ATP has no effect. Fusion is inhibited by K-glutamate, and several organic and inorganic cations and anions. These conditions are quite different from those reported for promotion and inhibition of exocytosis of granule contents from permeabilized chromaffin cells (2). We conclude that the membrane fusion seen here is activated by a different mechanism.

The protein synexin lowers the Ca^{2+}-K_m for aggregation to 200 μM (3) but also aggregates and fuses a variety of artificial phospholipid membranes (3,4). Synexin II, also isolated from adrenal medulla and liver, has similar Ca^{2+}-specific aggregation activities although it shares no peptides with Synexin I and shows qualitatively different aggregation kinetics (5). Calelectrin, isolated from the same sources, will aggregate granules (6). We have tested the ability of several proteins to perform these functions, which question the specificity of synexin(s) in exocytosis.

1. Morris et al, J Autonomic Nerv Sys 7 (1983) 19-33. 2. Knight and Baker, J Memb Biol 68 (1982) 107-140. Creutz et al, JBC 253 (1978) 2858-2866. 4. Morris and Hughes, BBRC 91 (1979) 345-350. 5. Hong et al, JBC 256 (1981) 3641-3644. 6. Odenwald and Morris, BBRC 112 (1982) 147-154. 7. Sudhof et al, Biochemistry 23 (1984) 1103-1109.

340.7 A NEW IMMUNOLOGICAL APPROACH TO THE FRACTIONATION OF PHOTORECEPTOR MEMBRANES: IMMUNOAFFINITY PARTITIONING. A.S. Polans, L. Labieniec Pintel*, S.D. Flanagan# and D.S. Papermaster*. Dept. of Pathology, VA Medical Center, West Haven, CT 06516, Yale Medical School, New Haven, CT 06510 and #Div. of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Immunoaffinity partitioning is a method by which membranes are selectively fractionated dependent upon their unique antigenic composition. Membranes are incubated sequentially with primary antibody (Ab) of defined specificity, biotinylated antiimmunoglobulin (BtnAb) and finally an aqueous polymer system consisting of 4.3% dextran and 3.6% polyethylene oxide [poly(EtO)] in buffer. The two polymers separate into a top, poly(EtO)-rich phase and a bottom, dextran-rich phase. Streptavidin, a biotin binding protein, is conjugated to poly(EtO) [SAvpoly(EtO)] and added to the phase mixture. A bridge between membrane-Ab-BtnAb-SAvpoly(EtO) is formed, thus partitioning the specific membrane to the top phase.

Monoclonal Ab 1D4 (a gift of R. Molday, U.B.C.) binds to the C-terminal of opsin, exposed to the cytoplasmic surface of the disc membrane, and partitions 70-80% of biosynthetically labeled frog rod outer segment membranes (ROS) to the top phase. In the absence of 1D4 or other bridge components, ROS partition to the bottom phase or interface. Nonimmune IgG in place of 1D4 or excess free biotin blocks partitioning, thus demonstrating the specificity of the bridging reactions. In contrast to 1D4, a sheep antiopsin antibody which binds mostly to the N-terminal of opsin, in the interior of the disc, partitions ROS only upon disruption of the membrane by detergents or storage at 4°C. Immunoaffinity partitioning therefore not only fractionates membranes, but is a simple method for assessing the orientation of a molecule within the membrane. To this end we are preparing antibodies to peptide sequences of opsin and are determining their efficacy in partitioning various ROS preparations. The dependence of partitioning upon the physical state of the membrane, antigen density and factors such as divalent cation concentration also is being investigated.

Our objective is to fractionate photoreceptor membranes and address questions of molecular sorting and how the resultant distribution of molecules relates to function. Supported by USPHS grants EY-00845, RR-05841, NS-18854 and the Veterans Administration.

340.8 THERMOTROPIC BEHAVIOR OF BINARY MIXTURES OF GLYCOSPHINGOLIPIDS WITH DIPALMITOYLPHOSPHATIDYLCHOLINE. B. Maggio*, T. Ariga*, J.M. Sturtevant*, R.K. Yu. Departments of Neurology & Chemistry. Yale University. CT 06510.

To gain a better understanding of the properties and organization of glycosphingolipids on cell membrane surface, we have recently studied by high sensitivity differential scanning calorimetry the thermotropic behavior of binary mixtures of dipalmitoylphosphatidylcholine (DPPC) with neutral (GalCer, GlcCer, phrenosine, kerasine, Gg_4Cer) or anionic (sulfatide, GM_3, GM_1, GD_{1a}) glycosphingolipids over the entire composition range. In all systems, the pretransition of DPPC was completely abolished and the cooperativity of the main transition decreased sharply at glycolipid molar fractions between 0.1-0.2. All mixed systems revealed complex non-ideal phase diagrams. Regions of isothermal melting, indicating lateral phase separation in the gel and liquid-crystalline phases, were found for mixtures of DPPC with GalCer, GlcCer, phrenosine or sulfatide. This monotectic behavior was present on the glycolipid-rich side of the temperature-composition diagram; complete miscibility in both the solid and liquid phases was found in the DPPC-rich region. Systems constituted with Gg_4Cer or gangliosides showed almost complete miscibility except for glycosphingolipids at molar fractions of less than 0.05 where solid phase immiscibility was probably present. At low proportions of glycosphingolipids, the systems constituted with GalCer, GlcCer, phrenosine, kerasine, sulfatide or GD_{1a} were shifted to temperatures lower than those expected for the ideal systems. As the molar fraction of the glycosphingolipid was increased, the deviation from the ideal behavior was reduced, except for the mixtures of DPPC and GD_{1a} for which the opposite was observed. For mixtures of DPPC with gangliosides GM_3 or GM_1 the systems had higher temperatures than those expected for the ideal systems. The calorimetric transition enthalpies of mixtures of DPPC with neutral glycosphingolipids were lower than those expected for an ideal system. Conversely, the transition enthalpies were higher than in the ideal case for gangliosides-DPPC systems. Thus, a new transition process occurred in the latter mixtures at molar fractions of gangliosides between 0.1 and 0.6, depending on the particular ganglioside. (Supported by NIH Grants NS-11853, GM-04725, NSF Grant PCM-8117341 and NMSS Grants RG 1289-B-3 and FG 644-A-1).

340.9 **DOES PROTEIN KINASE C ACTIVITY REGULATE NEURAL PLASTICITY AND ITS TIME-DEPENDENT PROCESSES? R.B. Nelson*, R.F. Akers*, and A. Routtenberg. (Spon: S. Chan) Cresap Neuroscience Laboratory, Northwestern University, Evanston IL 60201.**

The molecular regulation of neural plasticity should reflect the different temporal requirements of this process. Our laboratory has implicated the phosphorylation of Protein F1 (MW 47kD, IEP 4.5) in such plasticity (Fed.Proc. 42, p.755, 1983; Behav.Neur.Biol. 1984). Protein F1 *in vitro* phosphorylation is increased in rat hippocampal formation 5 min. after the induction of long-term potentiation (LTP), or 3 days following long-term enhancement (LTE; Lovinger et al., this meeting). Protein F1 kinase is stimulated by Ca2+ and phosphatidylserine, but not by calmodulin or cAMP. Moreover, Protein F1 serves as a substrate for exogenously added Ca++ and phospholipid dependent protein kinase C (Takai et al., J.Biol.Chem. 1977) but not for calmodulin-dependent kinases. We propose that Protein F1 phosphorylation is stimulated following LTP and LTE by the physiological activation of kinase C. It is further suggested that previously described mechanisms of kinase C activation may regulate the different durations of these forms of plasticity. For example, kinase C is *transiently* activated by diacylglycerol formation or Ca++. More *prolonged* activation of kinase C occurs by (a) translocation of the enzyme from cytosol to membrane, and (b) proteolytically-directed liberation of the enzyme's catalytic subunit from its regulatory subunit. This proposal suggests the possibility that post-translational mechanisms of protein modification not involving *de novo* protein synthesis may be sufficient for the regulation of neural plasticity with different temporal domains. (Supported by MH25281 and AFOSR 83-0335 to A.R.)

340.10 CALCIUM-DEPENDENT, GANGLIOSIDE STIMULATED PHOSPHORYLATION IN RAT BRAIN MEMBRANE. R.K. Yu, J.R. Goldenring, L.C. Otis* and R.J. DeLorenzo. (SPON.: Z. Smith) Dept of Neurology, Yale Univ. School of Medicine, New Haven CT 06510.

Calcium ion has been recognized as an important regulator of phosphorylation through its interaction with calmodulin-dependent and phospholipid-dependent kinases. Gangliosides are complex glycolipids which have high affinity for calcium ion. In the presence of calcium ion, a bovine brain ganglioside mixture (with major components GM1, GD1a, GD1b, and GT1b)stimulates the phosphorylation of a number of rat brain membrane proteins with molecular weights of approximately 42K Da, 50K Da, 60K Da, 80K Da, 140K Da and higher molecular weight species. Stimulation of phosphorylation by gangliosides was totally dependent on the presence of calcium ion. However, in the absence of added calcium ion, the calcium salt of bovine brain gangliosides prepared by extensive dialysis fully activated the membrane phosphorylation. Individual gangliosides were tested for stimulation of membrane phosphorylation with the following values for half maximal stimulation: GT1b, 24 uM; GD1a, 33 uM; GD1b, 25 uM; and GM1, 150 uM. Sulfatide, asialo-GM1, cerebroside, sialic acid, phosphatydylserine and phosphatidylinositol did not elicit any stimulation of membrane phosphorylation at any concentrations. Thus, stimulation required both the lipid and sialic acid portions of gangliosides, with disialo and trisialo gangliosides displaying higher stimulatory capacity. The pattern of calcium-dependent ganglioside stimulated phosphorylation was qualitatively similar to that observed with calcium-calmodulin-dependent phosphorylation. However, while calmodulin-dependent phosphorylation was inhibited with an IC(50) of approximately 5 uM trifluoperazine, ganglioside stimulated phosphorylation was inhibited with a much higher IC(50) of 500 uM trifluoperazine. This inhibition of ganglioside stimulated phosphorylation at relatively high concentrations of trifluoperazine is indicative of inhibition of general lipophilic properties. No calcium-dependent ganglioside stimulated phosphorylation was observed in the presence of GTP rather than ATP. The data indicate that gangliosides can stimulate rat membrane phosphorylation following the binding of calcium ion to the ganglioside molecules suggesting that calcium-ganglioside interaction may have an important role in regulating membrane processes.

340.11 GLYCOSPHINGOLIPID BIOSYNTHESIS DURING MYOGENESIS IN VITRO: A COMPARATIVE STUDY. P.E. Buse*, E.L. Hogan and K.C. Leskawa*. Department of Neurology, The Medical University of South Carolina, Charleston, SC 29425

Previously, we reported stage-specific changes in the biosynthesis of membrane glycosphingolipids (GSLs) during the differentiation of chick embryo muscle cells in vitro (Hogan, Chien and Leskawa, Soc. Neurosci., Boston, MA, 1983). During the initial stages of myoblast fusion, significant increases in biosynthesis of triosylceramide and the Forssman hapten were observed. Now we have similarly analyzed three other in vitro myogenic models: the rat L6 muscle cell line and two clones isolated from dissociated mouse hind limb muscle, the G-7 and the G-8 lines. Cells in culture were divided into four myogenic stages: a replicating stage (R), when single myoblasts are actively dividing; confluency (C), when cell membranes become opposed; post-confluency (PC), when muscle cells initiate fusion; and a final phase where multinucleated myotubules predominate (MT). GSL biosynthesis was assessed using 3H-galactose as a precursor, the products being resolved by TLC and autoradiography.

Rat L6 cells biosynthesized large amounts of ganglioside GM3 during all phases. Major neutral GSL products were lactosylceramide and paragloboside (nLcOse4Cer). Few significant changes during myogenesis were observed.

Mouse G-7 myoblasts biosynthesized GlcCer, LacCer, CTH (GbOse3Cer), nLcOse4Cer and nLcOse5Cer during the R phase. Upon confluency, biosynthesis of GlcCer increased and nLcOse5Cer biosynthesis stopped. When the cells began fusing (PC), GlcCer biosynthesis abruptly ended. G-7 myotubules (MT) biosynthesize predominantly LacCer. Changes in ganglioside biosynthesis included a decrease in GM3 during myogenesis, and increases in GD1a and 2 gangliosides of the paragloboside series, MG-IV and MG-VI.

Mouse G-8 myoblasts biosynthesized nLcOse4Cer as the major neutral GSL during replication (R). During myogenesis, there were significant increases in LacCer and GbOse3Cer. As with G-7 cells, MG-IV biosynthesis increased with myogenesis, but GM3 and MG-VI did not change significantly.

These results substantiate that there are stage-specific alterations in GSL biosynthesis during muscle cell differentiation, but these changes are not universal and are unique to each in vitro myogenic model.

Supported by the Muscular Dystrophy Association.

340.12 Na^+, K^+-ATPase AND Ca^{2+}, Mg^{2+}-ATPase ACTIVITY IN KIDNEY TISSUES OF DAHL RATS. J.H. Rho and Y.Y.T. Teng*. Department of Medicine, Schools of Medicine and Pharmacy, University of Southern California, Los Angeles, CA 90033

Both Dahl salt-sensitive and salt-resistant rats were split into two different dietary groups and were fed a diet with either 0.4% or 8.94% NaCl for 3 to 4 weeks. The detergent 3-(3-cholamidopropyl dimethylammonio)-1-propane sulfonate (CHAPS)-purified membrane fractions of outer medulla portion of Dahl rats from each diet group were prepared by the procedure described by Jorgenson (Biochim. Biophys. Acta 356, 36-52, 1974), and the ATPase activities were assayed according to the procedure described by Glynn and Kralish (in Membrane Adenosine Triphosphatases and Transport Processes. Ed. R. Bronk, London, Biochem Soc. 1974).

Mean values for the four groups are expressed as μ moles of inorganic phosphorus hydrolyzed per mg protein per minute of incubation time. A high salt diet does stimulate the Na^+, K^+-ATPase activities of the kidney in both strains from 23 to 25% but the enzymatic activity of the salt-sensitive strain on a high salt diet is about 14% less ($p < 0.05$) than that of the salt-resistant strain.

In contrast the Ca^{2+}, Mg^{2+}-ATPase activity of Dahl salt-sensitive rats was highly enhanced (24.7%) by a high salt diet in comparison to that of Dahl salt-resistant rats (8.6%) on the same high salt diet. Such differential enhancement of the Ca^{2+}, Mg^{2+}-ATPase activity in Dahl rats may be resulted as an adaptive mechanism to increased intracellular Ca^{2+} levels in the salt-sensitive rats.

340.13 EFFECTS OF GLIAL CELL DEFICIENCY ON DEVELOPING AXOLEMMA OF RAT DORSAL FUNICULUS. J.A. Black*, T.J. Sims, S.G. Waxman and S.A. Gilmore. Dept. of Neurology, Stanford Univ. Sch. of Med., V.A. Med. Ctr., Palo Alto, CA 94305 and Dept. Anatomy, Univ. of Arkansas for Med. Sc., Little Rock, AR 72205.

Irradiation of the lumbosacral spinal cord of 3-day-old rat with 4000 r profoundly decreases the developing glial cell population, while sparing neuronal elements. At birth, axons of the rat dorsal funiculus (DF) are essentially all non-myelinated. Numerous myelinated fibers are present by 14 days in normal rats. Irradiation at 3-days of age results in a consistent reduction in oligodendrocytes and astrocytes, while axons do not appear to be effected by this procedure. Thus, the structure of developing axolemma may be studied in the absence of normal axo-glial interactions.

Control and irradiated 19-day-old rats were perfused with 2% glutaraldehyde and 2% paraformaldehyde in 0.14 M phosphate buffer. Tissue for thin section and freeze-fracture investigation was processed according to conventional methods (Black et al., 1981).

At 19-days of age, the DF of control animals are well-populated with myelinated fibers. Most large caliber axons (>1 μm diameter) are myelinated at this stage, and numerous smaller caliber axons remain non-myelinated. In contrast, the DF of irradiated animals at 19-days of age have few fibers that are myelinated, despite the presence of numerous large caliber axons. The number of fibers within the DF does not appear to be altered by irradiation.

Analysis of freeze-fracture replicas of control non-myelinated (C n-m) and internodal (C in) axolemma, and irradiated (I o) axolemma devoid of myelin, reveal the following intramembranous particle (IMP) densities (mean±SEM):

Condition	P-face (IMP/μm²)	E-face (IMP/μm²)
C n-m	704±82.4	542±62.2
C in	2015±251.6	123±14.0
I o	1218±133.4	295±44.2

Plots of apparent axonal diameter versus IMP density for irradiated fibers (I o) in the absence of myelin disclose that, on the P-face, increasing axonal diameter is accompanied by increasing IMP density. For these fibers, large axons (>1 μm dia.) have a high IMP density (2258±198.6), whereas smaller fibers (<0.5 μm dia.) have a much lower IMP density (922±119.9). These data suggest that axolemmal differentiation may occur in the absence of glial interaction. (Supported by: SGW-NIH NS-15320, NMSS RG-1231, and Medical Res. Svc., VA; SAG-NIH NS-04761)

340.14 Glutamate Receptor Regulation by the Cytoskeletal Protein Fodrin. R. Siman, M. Baudry, and G. Lynch. Dept. of Psychobiology, Univ. of California, Irvine, Ca. 92717.

The most recently described cytoskeletal system of neurons is a submembraneous lining consisting of the rod-shaped protein fodrin. In many of its properties, fodrin closely resembles spectrin, the principal component of the cytoskeleton of erythrocytes. Spectrin is known to exert transmembrane control over cell surface proteins, but the functional role of neuronal fodrin is less clear. We tested the possibility that fodrin regulates the distribution of synaptic membrane proteins, in a manner similar to its erythrocyte counterpart. We focused on a possible interaction between fodrin and binding sites for the putative neurotransmitter L-glutamate, since the number of glutamate binding sites increases following activation of the calcium-stimulated protease calpain I, an enzyme for which fodrin is a substrate.

A fodrin-glutamate binding site interaction was tested for by examining the effect of antibodies to purified rat brain fodrin on Na^+-independent glutamate binding to rat brain synaptic membranes. Antifodrin did not alter glutamate binding to hippocampal or cortical membranes measured in Tris-HCl buffer. However, antifodrin completely prevented the increase in glutamate binding induced by micromolar calcium concentrations. This blockade of Ca^{2+}-stimulated glutamate binding did not result from calcium chelation by antifodrin and was not observed with pre-immune serum. Antifodrin was effective when added before but not after calcium. Thus antifodrin does not directly modify glutamate binding but rather interferes with the process by which calcium increases binding. The blockade of Ca^{2+}-stimulated glutamate binding did not depend on fodrin cross-linking since monovalent Fab fragments were equally effective. The dose-response for antifodrin prevention of Ca^{2+}-stimulated glutamate binding was virtually identical to that for inhibition of (^{125}I)-antifodrin binding to synaptic membranes, indicating a strong correlation between the capacity of antifodrin to attach to fodrin and to block Ca^{2+}-stimulated glutamate binding. Fodrin thus exerts transmembrane control over synaptic membrane proteins. Those doses of antifodrin that blocked Ca^{2+}-stimulated glutamate binding also inhibited Ca^{2+}-induced fodrin proteolysis. These results suggest that calcium-induced proteolysis of fodrin leads to the increase in glutamate binding site density. Modification of the fodrin cytoskeleton by calcium could be a molecular mechanism that underlies various forms of synaptic plasticity.

ACETYLCHOLINE II

341.1 ACTION OF ACETYLCHOLINE AND MUSCARINE ON NEURONS OF CAT SENSORIMOTOR CORTEX IN VITRO. M.C. Chubb*, P.C. Schwindt and W.E. Crill (SPON: P.D. Swanson). VA Med. Ctr., Seattle, WA 98108, and Depts. Physiol. Biophys. and Medicine (Neurol.), Univ. of Washington Sch. of Med., Seattle, WA 98195.

The action of acetylcholine (Ach) and muscarine (Musc) on neurons in layer V of cat sensorimotor cortex were studied in brain slices using current clamp and single microelectrode voltage clamp. Ach and Musc were applied either as droplets to the slice surface (5-100 mM in saline) or in the perfusate (10 μM) with equivalent results. The depolarization produced by either agent was usually modest (ca. 5 mV), but a marked change in firing behavior was always apparent. The slow afterhyperpolarization components that follow a period of repetitive firing in these cells (see abstract by Schwindt, Spain, Stafstrom, Chubb and Crill, this volume) were replaced by a prolonged afterdepolarization (ADP) of variable amplitude lasting up to several seconds. If initially subthreshold, the ADP could be made to "summate" by repetitive stimulation, and to reach spike threshold in some cells. After the ADP reached threshold, repetitive firing commenced in the absence of further stimulation and could be halted only temporarily during the application of hyperpolarizing injected current pulses. If Musc was applied following tetrodotoxin (TTX), the Musc-dependent ADP could still be evoked by strong, prolonged depolarization; sometimes, repetitive Ca^{++} spikes (cobalt sensitive) occurred during and persisted after the depolarization, an effect never seen in the presence of TTX alone. Application of TTX and 10 mM tetraethylammonium also resulted in the appearance of repetitive Ca^{++} spikes during sufficient depolarization. Under these conditions, following repetitive Ca^{++} spikes, the Musc-dependent ADP could reach 20 mV amplitude and last up to 80 sec. Two Musc-sensitive outward current components were identified in cells examined under voltage clamp. One component had slow onset and was first activated strongly ca. 30 mV positive to RP. This component resembles the m-current of Brown and Adams (Nature, 1980). The onset of the other component was too fast for the single electrode voltage clamp to measure accurately. Although compatible with altered firing behavior, the depression of outward currents is insufficient to account for the appearance of the Musc-dependent ADP, which requires the presence of an inward ionic current near resting potential. It is hypothesized that the ADP is generated in part by inward currents resulting from prolonged, Ca^{++}-dependent depolarization of dendrites secondary to depression of outward currents by Musc and initiated by adequate somatic depolarization.

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341.2 **CHOLINERGIC STIMULATION OF GLIAL CELL ARACHIDONIC ACID AND PHOSPHATIDIC ACID METABOLISM.** **J.J. DeGeorge*[1], P. Morell[2], K. McCarthy*[3], and E. Lapetina*[4].**
Univ. of North Carolina at Chapel Hill, Biol. Sci. Res. Ctr.[1,2] and Dept. of Biochem.[2], and Pharm.[3], Chapel Hill, N.C. 27514 and The Wellcome Research Laboratories Dept. of Mol. Biol.[4], RTP, N.C. 27709.

The potential involvement of arachidonic acid (AA) metabolites in neurotransmission within the central nervous system (CNS) has received recent attention (for review; Wolf, L., J. Neurochem. 38:1, 1982). We have examined the effects of cholinergic stimulation on the metabolism of endogenous AA by glia using C62B cells as a model. When C62B cells were incubated for 18 hr with 6 μM [1-^{14}C] AA in the presence of 5% fetal bovine serum more than 85% of the radioactivity was incorporated into cellular lipids; less than 3% of the remaining free [1-^{14}C] AA was cell associated. Treatment of the labeled cells with acetylcholine or its stable analog carbachol stimulated the liberation of [1-^{14}C] AA from esterified pools. The levels of liberated AA peaked within 2 min and returned to basal levels by 5 min. This process was dose dependent over the range of 0.01 - 5.0 mM and was selectively blocked by the muscarinic antagonist atropine, but unaltered by the nicotinic antagonist d-tubocurarine. The increased liberation of [1-^{14}C] AA was accompanied by an increase of [1-^{14}C] AA labeled phosphatidic acid and the formation of a [1-^{14}C] AA metabolite (tentatively identified as the lipoxygenase product 5,12 HETE). The liberation of AA and the formation of 5,12 HETE were blocked by drugs which inhibit phospholipase A2 activity (trifluoperazine or mepacrine). Thus, stimulation of muscarinic cholinergic receptors on C62B cells is associated with: 1) increased formation of phosphatidic acid, suggesting activation of phospholipase C, 2) liberation of AA from cellular phospholipid stores via activation of phospholipases of the A2 type, and 3) metabolism of part of this liberated AA to hydroxylated derivatives know to regulate cell function in other cell types. Experiments are presently in progress to determine if cholinergic stimulation of primary glial cells elicits a similar cascade of AA metabolism, and to examine the effects of the AA metabolites formed on glial and neuronal function. Supported by USPHS grants NS07166, NS11615, HD03110 and NS20212.

341.3 CHOLINERGIC PATHWAYS II: BASAL FOREBRAIN AND PONTINE TEGMENTUM INNERVATE THE INTERPEDUNCULAR NUCLEUS IN THE RAT. L. L. Butcher, N. J. Woolf, and F. Eckenstein. Brain Research Institute and Dept. of Psychology, University of California, Los Angeles, CA 90024.

Simultaneous visualization of choline-O-acetyltransferase (ChAT)-like immunoreactivity and retrogradely transported fluorescent propidium iodide (PI) in numerous cells of the basal forebrain and pontine tegmentum delineates at least two significant sources of cholinergic input to the interpedncular nucleus and surrounding ventral tegmental area.

Following tracer infusions into the interpeduncular nucleus PI-labelled somata in the basal forebrain that were also immunoreactive for ChAT were preferentially found in the horizontal limb of the diagonal band (for terminology, see Bigl et al., Brain Res. Bull., 8:727, 1982), magnocellular preoptic area, and substantia innominata. In some parts of the horizontal limb of the diagonal band and the magnocellular preoptic area greater than 50% of the ChAT-labelled cells appeared to provide afferents to the interpeduncular nucleus. A few cholinergic projection cells were also found in the vertical limb of the diagonal band and in nucleus basalis. PI-labelled cells that were not reactive for ChAT were also observed in the vertical and horizontal limbs of the diagonal band, magnocellular preoptic area, and substantia innominata.

Many PI-labelled cells were found in the dorsolateral tegmental nucleus following infusions of tracer into the interpeduncular nucleus. Approximately half of the PI-labelled cells in the dorsolateral tegmental nucleus contained ChAT. Furthermore, the majority of ChAT-containing cells at caudal levels of the dorsolateral tegmental nucleus were PI-labelled. A few ChAT and PI co-labelled cells were also found in the caudal parts of the pedunculopontine tegmental nucleus.

The interpeduncular nucleus receives input from both the cholinergic basal forebrain and the cholinergic pontine tegmentum. This is unlike most brain regions (e.g., neocortex, thalamus) which appear to be innervated preferentially from one or the other cholinergic projection system.

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341.4 CHOLINERGIC PATHWAYS III: PROJECTIONS FROM THE CHOLINERGIC PONTINE TEGMENTUM TO THE THALAMUS, TECTUM, BASAL FOREBRAIN, AND BASAL GANGLIA OF THE RAT. S. R. McGurk, N. J. Woolf, F. Eckenstein, and L. L. Butcher. Brain Research Institute and Dept. of Psychology, University of California, Los Angeles, CA 90024.

Biochemical studies have indicated that the thalamus and the tectum receive cholinergic inputs from the midbrain reticular formation (Hoover & Jacobowitz, Brain Res., 170:113, 1979). Data in the present report indicate that such cholinergic projections derive from a group of choline-O-acetyltransferase (ChAT)-containing and acetylcholinesterase (AChE)-intense neurons in the pedunculopontine tegmental nucleus and the dorsolateral tegmental nucleus. The cholinergic pontine tegmentum also was found to innervate certain basal forebrain areas and basal ganglionic structures.

The fluorescent tracers Evans Blue (EB) and propidium iodide (PI) were infused into various sites in the thalamus, tectum, basal forebrain, and basal ganglia. Brains infused with EB were subsequently stained for AChE according to the pharmacohistochemical method (see Butcher, in Handbook of Chemical Neuroanatomy, 1:3, 1983, Elsevier) and brains infused with PI were reacted with antisera directed against ChAT.

ChAT-labelled and AChE-intense neurons in the pedunculopontine tegmental nucleus projected predominantly to the midline and posterior thalamic nuclei, superior colliculus, globus pallidus, entopeduncular nucleus, and subthalamic nucleus. Only a few cholinergic pedunculopontine tegmental neurons provided inputs to anterior thalamic nuclei and certain basal forebrain areas. The cholinergic neurons of the dorsolateral tegmental nucleus contributed largely to the innervation of the following basal forebrain areas: the medial septal/diagonal band region, magnocellular preoptic area, and lateral hypothalamus, as well as to the anterior and midline thalamus.

The results of the present study suggest that the ascending projections of the cholinergic pontine tegmentum are more widespread than previously believed. Furthermore, a rough topography was observed for these ascending cholinergic projections with the dorsolateral tegmental nucleus innervating more rostral structures and the pedunculopontine tegmental nucleus more caudal areas.

[Support: NS-10928 to L.L.B.]

341.5 CHOLINERGIC PATHWAYS I: PROJECTIONS FROM THE BASAL FOREBRAIN TO THE LIMBIC TELENCEPHALON IN THE RAT. N. J. Woolf, F. Eckenstein, and L. L. Butcher. Brain Research Institute and Dept. of Psychology, University of California, Los Angeles, CA 90024.

Cholinergic projections to regions of the limbic telencephalon were studied by microscopically assessing the cellular co-localization of the fluorescent tracer propidium iodide (PI) and immunohistochemically demonstrated choline-O-acetyltransferase (ChAT). PI was infused into the olfactory bulb, amygdala, hippocampus, subiculum, and the entorhinal, pyriform, cingulate, and insular cortices.

Numerous neurons in the medial septal nucleus and the vertical and horizontal limbs of the diagonal band (for basal forebrain terminology used, see Bigl et al., Brain Res. Bull., 8:727, 1982) demonstrated projections to the hippocampus. Of these over 60% also contained ChAT. Basal forebrain neurons that projected to the olfactory bulb were found in the horizontal limb of the diagonal band and the magnocellular preoptic area; less than 10% of the basal forebrain cells projecting to the olfactory bulb were immunoreactive for ChAT. The distribution of PI-labelled, ChAT-positive cells projecting to the amygdala was similar to that reported previously (Woolf & Butcher, Brain Res. Bull., 8:751, 1982).

The horizontal limb of the diagonal band contained cells co-labelled for PI and ChAT following infusions of tracer into the subiculum and entorhinal cortices.

The cingulate cortex received ChAT-containing projections from the magnocellular preoptic area, the substantia innominata, and the nucleus basalis. Almost all of the basal forebrain cells that projected to the cingulate cortex contained ChAT. Pyriform and insular cortices received similar projections from ChAT-positive cells in the horizontal limb of the diagonal band, magnocellular preoptic area, substantia innominata, and the nucleus basalis. The caudal magnocellular preoptic area contained PI-labelled cells following pyriform cortex infusions that were not co-labelled for ChAT.

As intimated above, the cholinergic innervation of the limbic cortex is organized in a crude topographic fashion. The most rostral cells in the cholinergic basal forebrain preferentially projected to allocortical regions, whereas those more caudally situated preferentially innervated insular and proisocortical fields.

[Support: NS-10928 to L.L.B.]

351.6 CEREBROSPINAL FLUID (CSF) CHOLINE LEVELS ARE ELEVATED IN YOUNG ADULT DOWN SYNDROME SUBJECTS. J. Hodes,* A. Kay,* M. Schapiro,* I. Hanin, U. Kopp,* S. Rapoport, N. Cutler (SPON: C. Grady) Laboratory of Neuroscience, NIA, NIH, 10/6C103 Bethesda, Md. 20205 and U. of Pittsburgh, Western Psychiatric Institute and Clinic, 3801 O'Hara St., Pittsburgh, PA. 15261

Down syndrome (DS) is the most common congenital abnormality with known etiology, affecting the brain and causing mental retardation. Brains of young adult DS subjects show no consistent abnormalities, but brains of DS subjects, older than 35 years, show senile plaques, neurofibrillary tangles, granulovacular degeneration, and reduced activity of choline acetyltransferase and acetylcholinesterase.

We examined choline concentrations (conc) in CSF from 13 (8 M, 5 F) DS subjects and 15 M normal controls to see if this putative marker of CNS cholinergic function was altered.

Male volunteers, 5 aged 20-35 yr (mean 27) and 10 aged 61-77 yr (mean=70) were screened for the absence of primary or secondary brain disease or for conditions that might contribute to brain dysfunction. As well, 10 DS subjects with proven trisomy 21 [5 M, 5 F] aged 21-34 yr (mean=27) and 3 M aged 47-63 yr (mean 55) were similarly screened. Subjects were admitted to the ward 3 days prior to lumbar puncture (LP) and fed a diet low in monoaminergic precursors. Subjects were off medications for 2 wks. LP was performed in the lateral recumbant position between 08:30 - 10:00 following 8-10 hours of strict bedrest. CSF samples were collected, the first 12 cc were pooled and 1 cc aliquots were frozen and stored at -70^{0}C until choline det. Choline assay was performed on GC/MS according to Hanin and Skinner, Anal. Biochem., 66, 1975.

Normal subjects showed age dependent increases in CSF choline conc ($r=0.76$; $p<0.01$). As well, the young (20-35 yr) DS subjects showed significantly higher ($p<0.05$) CSF choline conc (3.4±SD 0.59 nmoles/ml) than the young normals (2.4±SD 0.39 nmoles/ml). No significant differences in CSF choline conc were detected in the 2 older groups.

Elevations of CSF choline conc in young DS subjects may reflect increases in the activity of the central cholinergic system. This finding is consistant with the report of increased cerebral glucose utilization in DS (Schwartz et al., Science 221, 1983). Perhaps an increased global cerebral metabolic rate in these subjects may be responsible for the apparent increases in choline conc. Grant# MH 26320 to I.H.

341.7 CHOLINERGIC AND OPIATE INVOLVEMENT IN THE ANTINOCICEPTIVE EFFECT OF DIISOPROPYLFLUOROPHOSPHATE. L.G. Costa, and S.D. Murphy*. Department of Environmental Health, University of Washington, Seattle, WA 98195.

Previous studies (Koehn et al, Eur. J. Pharmacol. 61, 167, 1980; Zorn, Costa, and Murphy, Toxicologist 3, 14, 1983; 4, 171, 1984) have shown that the acetylcholinesterase inhibitor diisopropylfluorophosphate (DFP) exerts an antinociceptive effect in laboratory animals, which is antagonized by muscarinic antagonists and by the opiate antagonist naloxone. This suggests an involvement of both the cholinergic and the opiate system in DFP-induced antinociception. In the present study we have further investigated the antinociceptive effect of DFP using a tail-immersion test. DFP (3 or 6 mg/kg, ip, in corn oil) caused a dose dependent antinociception in mice which was antagonized by the muscarinic antagonist scopolamine (1 mg/kg.) Naloxone (2 mg/kg) antagonized the antinociceptive effect of the highest dose of DFP (6 mg/kg) but did not affect the antinociception caused by 3 mg/kg DFP. The hypothermic effect of both doses of DFP was antagonized by scopolamine but not by naloxone. Twenty-four hours after the administration of DFP, colonic temperature had returned to control values in mice treated with 3 or 6 mg/kg DFP. Reaction time in animals which received 3 mg/kg DFP did not differ from control. However, reaction time was still significantly higher than control in mice administered 6 mg/kg DFP 24h earlier. Administration of naloxone 24 h after 6 mg/kg DFP antagonized this residual antinociceptive effect, while scopolamine caused only a slight reduction in reaction time.

These results suggest that antinociception induced by a low dose of DFP is primarily due to a cholinergic mechanism, while higher doses appear to affect also the opiate system. Since we have shown (Zorn et al, 1984) that DFP (6 mg/kg) increases met-enkephalin levels in the brain, it is possible that higher doses of DFP might interfere with enkephalin metabolizing enzymes. This would agree with in vitro studies showing that higher concentrations of DFP are required to inhibit the peptidase activity of acetylcholinesterase (Chubb et al, Neurosci. 5,2065, 1980; 10, 1369, 1983) than its esterase activity (Supported in part by NIEHS grant ES 03424).

341.8 CHOLINERGIC (CH) RECEPTORS IN THE RAT OLFACTORY BULB: NICOTINIC (N) AND MUSCARINIC (M) CHOLINERGIC RECEPTORS ARE SEGREGATED AND COINCIDE WITH ACETYLCHOLINESTERASE (AChE). G. Blaha, W. Blair, W.T. Nickell and M.T. Shipley. University of Cincinnati College of Medicine, Cincinnati, OH 45267-0521.

We report that the adult rat olfactory bulb has a distinctive pattern of AChE staining. High levels are present in the glomerular layer, the deep half of the external plexiform layer (epl) and the internal plexiform layer (ipl). Levels are slightly lower in the superficial half of epl and in parts of the granule cell layer. There are no intrinsic cholinergic (Ch) neurons in the bulb; the major source of Ch input arises in the diagonal band (DB) (Macrides, et.al., '81; Van Ooteghem, et.al., '83). In DB at least 88% of all AChE + neurons contain ChAT (Eckenstein & Sofroniew, '83), strongly indicating that they are cholinergic. Anterograde transport of WGA-HRP iontophoresed into DB labeled fibers and terminals in ipl, epl and within the glomeruli.

Cholinergic receptor sites were localized using the autoradiographic technique of (Young and Kuhar, '79). The ligand for nicotinic (N) receptors was ^{125}I-α-bungarotoxin (α-BT); ligands for muscarinic (M) receptors were ^{3}H-scopolamine (Scop) and ^{3}H-QNB.

α-BT binding is almost exclusively restricted to the glomeruli. In sections incubated prior to fixation there was occasionally α-BT binding in the deep half of epl. By contrast, Scop and QNB binding was low in glomeruli and very high throughout epl and ipl with some binding in the granule cell layer (gcl).

These results suggest that nicotinic receptors are localized mainly in the glomeruli while muscarinic receptors are present in epl, ipl and in some parts of gcl. Such strict laminar segregation of N and M receptors within the same neural structure has not been reported for any other part of the brain. These results suggest that DB-Ch inputs may act upon two fundamentally different receptors ("fast" and "slow") at two different levels of functional processing in MOB. The distribution of N and M receptors precisely matches the distribution of AChE indicating that this enzyme is a reliable marker for Ch synapses in the bulb.

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341.9 AN INTRACELLULAR STUDY OF THE EFFECTS OF ACETYLCHOLINE ON NEONATAL RAT DORSAL HORN NEURONS IN VITRO. R.C. Ma* and N.J. Dun, Dept. of Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153

Intracellular recordings were obtained from dorsal horn neurons in the lamina III-V of the neonatal (5-18 days old) rat spinal cord slices. Superfusion of acetylcholine (ACh, 0.1-1 mM) caused a membrane depolarization in nearly every dorsal horn neuron tested and the response was markedly potentiated by the anti-cholinesterase agent, eserine (0.5 μM). The ACh-induced depolarization persisted in low Ca/high Mg solution. However, the excitatory potentials that occurred spontaneously in the dorsal horn cells were reversibly abolished by low Ca solution. Four types of cholinergic responses could be distinguished on the basis of their susceptibility to nicotinic and muscarinic antagonists. In the first type which represents the majority of neurons sampled, the ACh depolarization was completely and reversibly blocked by nicotinic antagonists hexamethonium (0.1 mM) or d-tubocurarine (50 μM), and irreversibly by α-bungarotoxin (1 μM). Second, the depolarization was markedly reduced by nicotinic antagonists and the residual response could be completely abolished by atropine (1 μM). Third, the ACh depolarization was greatly attenuated by atropine and the remaining small depolarization could be eliminated by nicotinic antagonists. Finally, muscarinic agonists caused a hyperpolarization which was reversibly blocked by low Ca/high Mg solution and tetrodotoxin (1 μM) in a very few cells sampled, suggesting that the response may be caused by the release of a second transmitter. The nicotinic depolarization was accompanied in most cases by a decrease of input resistance and hyperpolarization increased the response. Whereas, the muscarinic depolarization was associated with an increase of input resistance in a number of cells. The muscarinic hyperpolarization was associated with a decrease in membrane resistance and membrane hyperpolarization reduced the response. The results suggest that dorsal horn neurons are endowed with nicotinic and muscarinic receptors the distribution of which may vary from cell to cell, and the electrophysiological characteristics of the nicotinic and muscarinic depolarization or hyperpolarization are similar in many respects to those reported in the peripheral autonomic neurons. (Supported by NIH Grant NS 18710)

341.10 EFFECTS OF DIAZEPAM ON SOMAN AND ATROPINE INDUCED CHANGES IN LEVEL OF ACETYLCHOLINE IN RAT BRAIN AREAS. T.-M. Shih and B.A. Barney* (SPON: J.A. Romano). US Army Med. Res. Inst. Chem. Def., Aberdeen Proving Ground, MD 21010

In addition to atropine (ATR) and oximes, diazepam (DIA) has occassionally been added to the treatment regimen for anticholinesterase (AntiChE) poisoning, principally for its ability to suppress seizure activity and convulsions. Elevated levels of acetylcholine (ACh) induced by AntiChE in the central nervous system have been thought to be responsible for the seizure and convulsions. Although both soman (GD), a potent AntiChE, and ATR, a muscarinic antagonist, affect the cholinergic system, the actions produced by these two substances are markedly different. We have attemped to examine (1) the effects of DIA on ACh levels in the absence or presence of GD or ATR in discrete rat brain areas and (2) the action of DIA on the cholinergic system. Rats were treated with normal saline (0.5 ml/kg, s.c.), DIA (2.5 or 5.0 mg/kg, i.m.), GD (100 μg/kg, s.c.) and ATR (16 mg/kg, i.m.) alone or in combination. Thirty min after treatment, animals were killed by microwave radiation focused on the head. Brains were dissected into brainstem (B) cortex (C), hippocampus (H), midbrain (M) and striatum (S) and ACh was quantitatively analyzed by gas chromatograph/mass spectrometry. ACh was significantly increased by GD but decreased by ATR in brain areas C, H, M and S. DIA alone produced a dose-related increase of ACh in C, M and S; a decrease in B, and no change in H. Except in B, neither dose of DIA blocked the GD-induced ACh elevation, but both doses of DIA reversed the ATR-induced ACh reduction. Neither ATR alone nor DIA at a dose of 2.5 mg/kg plus ATR reversed the elevated ACh levels produced by GD. DIA, however, at 5.0 mg/kg, when combined with ATR and GD, did bring the ACh back to control levels. In all instances, the levels of ACh in the B of rats treated with the DIA were consistently and significantly lower than control. These data suggest that ACh levels are differentially affected by DIA in different brain regions, perhaps dependent on the density of GABAergic innervation. DIA does not affect increased ACh levels induced by GD, but reverses those changes produced by ATR, indicating a presynaptic action of DIA, either directly or indirectly, at cholinergic terminals. Reversal of GD induced ACh elevation by the high dose of DIA plus ATR might partially be responsible for DIA's anticonvulsive effects in AntiChE poisoning.

341.11 TOXICITY AND TISSUE CHOLINESTERASE ACTIVITY FOLLOWING SOMAN, ATROPINE AND HI-6 TREATMENT. T.A. Koviak*, T.-M. Shih, O.B. Smith*, A. Kaminskis*, and C.E. Whalley. US Army Med. Res. Inst. Chem. Def., Aberdeen Proving Ground, Maryland 21010.

Combined treatment with atropine sulfate (ATS) and HI-6 has been shown to be effective in protecting rats and mice from approximately 5 LD50 of organophosphorus soman intoxication. The primary antidotal action of HI-6 is thought to be due to reactivation of the soman-inhibited cholinesterase (ChE). We have conducted a series of studies in rats to evaluate the protective effects of HI-6 alone or in combination with ATS on both soman-induced toxicity and soman-induced depression of tissue ChE activity. In the 24-hr soman lethality study, treatment with HI-6 (125 mg/kg, i.p.) alone and ATS (16 mg/kg, i.m.) alone afforded protective ratios (PR) of 2.5 and 1.2, respectively. The combination of ATS plus HI-6 provided a PR of 5.5. In the ChE study, rats were killed 30 min post-treatment, and total ChE activity was analyzed by an automated colorimetric method. Soman (100 µg/kg, s.c.) produced a marked depression of ChE in all tissues studied, except in liver and duodenum, where no change from control values occurred. Treatment with HI-6 or ATS alone did not affect tissue ChE levels. The % of ChE inhibition following soman (100 ug/kg, s.c.) and soman plus HI-6 treatment, respectively, in the tissues were as follows: plasma 93.6, 53.2; red blood cells 87.4, 26.2; brainstem 75.2, 72.3; cerebral cortex 76.3, 70.2; hippocampus 86.1, 86.0; midbrain 75.0, 70.2; cerebellum 81.3, 77.0; striatum 56.6, 50.7; lung 95.4, 87.1; diaphragm 70.0, 38.0; intercostal muscle 53.8, 31.2; skeletal muscle 47.5, 23.0; heart 76.5, 69.2; aorta 65.7, 7.4; salivary gland 69.1, 36.4; adrenal gland 71.5, 59.3; kidney 59.7, 38.8; and spleen 54.4, 35.0. Addition of ATS to HI-6 treatment did not provide further reduction of ChE inhibition already afforded by HI-6 alone in soman treated tissues; the additional central protection provided by ATS administration was however important for survival. These results suggest that HI-6 alone can protect against soman toxicity to a certain degree, and that this protective effect of HI-6 may be attributed to its ability to reactivate soman inhibited peripheral tissue ChE acitivty.

341.12 RATE, PATTERN AND TIME-COURSE OF CARBACHOL-INDUCED PONTO-GENICULO-OCCIPITAL (PGO) WAVES. H.A. Baghdoyan, R.W. McCarley and J.A. Hobson. Lab. of Neurophysiol., Harvard Medical School, Boston, MA 02115.

Cholinergic microstimulation of the reticular formation (RF) produces a state characterized by the major electrographic and behavioral parameters of desynchronized (D) sleep. The induction of this state by microinjection of carbachol (Dcarb) is specific to the pontine RF, and the type of electrographic syndrome produced is dependent upon the site of drug administration within the pons. The purpose of this study is to quantitatively characterize the rate, pattern and time-course of PGO wave activity produced by carbachol microinjection into the peri-abducens region.

Cats were implanted with standard polygraphic recording electrodes for scoring state and stainless steel guide tubes aimed at the pontine RF for microinjection of carbachol (4ug in 500nl saline). Baseline (no injection) recordings served as controls. The PGO rate by behavioral state analysis showed that after carbachol, PGO frequency was increased during waking (W) from 0.01 waves/sec to 0.63 w/s and during D sleep from 0.50 w/s to 2.00 w/s. The pattern analysis revealed that: the number of PGO wave clusters ($\geq$ 3 waves) was increased from 142 during physiological D sleep to 412 during Dcarb (+190%), the no. of pairs was decreased from 100 to 40 (-60%) and the no. of single PGO waves was reduced from 234 to 34 (-85%). Pattern analysis also showed a regular alternation between clusters of high and low amplitude, with a mean number of 6.6 clusters alternating in sequence (range:2-23 clusters/sequence). Such regular alternations also were state-dependent, occurring during episodes of Dcarb but not during W or baseline. Time-course analysis was obtained by operationally defining an epoch from D end to D end (or Dcarb end to Dcarb end) and expressing the duration of each epoch as a percent of epoch completed (0-100%). Under control conditions PGO wave activity was maximal at 80% into the epoch and following administration of carbachol maximal PGO wave activity was reached by 50% into the epoch and remained at peak level until the end of the epoch.

These analyses provide the first quantitative data on PGO wave activity evoked by microstimulation of carbachol into the peri-abducens area. Further analyses of PGO activity produced by cholinergic microstimulation of more rostral pontine sites will provide important information about the optimal region for cholinergic evocation of D sleep.

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341.13 SPECIFIC BINDING OF [^{3}H]-HEMICHOLINIUM-3 TO RAT BRAIN MEMBRANES. K. Sandberg and J.T. Coyle. The Johns Hopkins Medical School, Dept. Psychiatry, Div. Child Psych., Baltimore, MD 21205.

Hemicholinium-3 (HCh-3) is a potent and specific inhibitor of the high affinity uptake process for choline localized on cholinergic neurons. In this investigation, the specific binding of [^{3}H]-HCh-3 (120 Ci/mmol; NEN) to crude syanptic membranes (Mb) prepared from rat brain was characterized. The ligand-Mb complex was isolated by rapid filtration with a Brandell Cell Harvester on glass fiber filters (#32; Scheichert and Schuell), which had been pre-soaked in 0.1% (v/v) polyethylenimine solution to reduce non-specific binding. Specific binding was defined as the total binding minus that occurring in the presence of 100 µM HCh-3.

Specific binding of [^{3}H]-HCh-3 (10 nM) reached equilibrium by 30 min at the pH optimum of 8.0 in 50 mM glycylglycine buffer containing 200 mM NaCl. The temperature optimum was 25°C and specific binding was abolished by prior heat denaturation of the Mb. Specific binding of [^{3}H]-HCh-3 exhibited tissue linearity between 200-600 µg protein. Under these conditions, the specific binding of [^{3}H]-HCh-3 to forebrain Mb was saturable and reversible; Scatchard analysis revealed a K_D of 36-40 nM, B_{max} of 312 fmoles/mg protein with a Hill coefficient of 1.0.

Choline displaced [^{3}H]-HCh-3 binding with a IC_{50} of 45 µM. At 100 µM concentration, the following inhibition of the specific binding of [^{3}H]-HCh-3 was observed with choline analogues: N-isopropylcholine (58% $\pm$ 6); N-butylcholine (35% $\pm$ 2); N-ethylcholine (59 $\pm$ 6); benzylcholine (3 $\pm$ 1%); this profile correlates with the potency of these analogues to inhibit [^{3}H]-choline uptake. The specific binding of [^{3}H]-HCh-3 exhibited an uneven regional distribution in the adult rat brain with the following values (pmoles/mg protein): striatum, 50.2 $\pm$ 6.9; hippocampus, 14.1 $\pm$ 2.3; cortex, 16.5 $\pm$ 3.2; cerebellum, 6.8 $\pm$ 1.1; midbrain-hypothalamus, 5.2 $\pm$ 1.0; pons-medulla, 3.9 $\pm$ 0.5.

These preliminary findings suggest that [^{3}H]-HCh-3 binds to the carrier-site mediating the high affinity uptake of choline on cholinergic neurons; thus, this ligand may be a useful probe for investigating the presynaptic component of cholinergic neurons.

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341.14 CHARACTERIZATION OF PHOSPHOLIPASES INVOLVED IN RECEPTOR-MEDIATED ARACHIDONIC ACID RELEASE. C. Forray, M. McKinney, and E. Richelson. Dept. Pharmacology and Psychiatry, Mayo Fdn. and Mayo Clinic, Rochester, MN 55905.

Recently, we demonstrated that in the murine neuroblastoma clone N1E-115, muscarinic (M1) and histamine (H1) receptors stimulate cyclic GMP formation through a mechanism that involves arachidonic acid release and metabolism by a lipooxygenase pathway (Snider et al., in press, PNAS). To gain insight into the sequence of events that leads from receptor activation to the release of arachidonic acid, we studied the localization and properties of phospholipase C(PLC) and A_2 (PLA_2). PLC activity was measured by the formation of [^{14}C]diacylglycerol (DAG) from 2-[^{14}C]-arachidonoyl phosphatidylinositol (PI) or the release of [^{3}H]m-inositol phosphate (IP) from [^{3}]PI. The highest specific activity of PLC was found in the 100,000 x g supernatant (10-fold vs. total cell homogenate). Optimal activity of the enzyme was obtained at pH 5.5 without detergents and at pH 7.4 in the presence of 1mM DOC. The enzyme was active in 1mM EDTA, but Ca^{++} greatly enhanced its activity (Km=0.3mM). Incubation of this cytosolic fraction of phosphatidylinositol 4-phosphate (PIP) or phosphatidylinositol 4,5-bis-phosphate (PIP_2) inhibited the formation of [^{14}C]DAG without lowering the total DAG formed, the order of preference being PIP_2<PIP<PI as estimated from the ratio of inositide/PI needed to inhibit by 50% the [^{14}C]-DAG formation. The 12,000 x g pellet contained substantial PLC activity, optimal at pH 7.4 without detergents; it had similar sensitivity to Ca^{++}. Soluble PLC activity was inhibited by mepacrine (MEP) (IC_{50} = 60µM) p-bromophenacylbromide (BPB) (IC_{50} = 35µM), and serine esterase inhibitors (SEI). The membrane bound PLC activity differed only in that much higher concentrations of MEP (5mM) were needed to obtain inhibition. PLA_2 activity was detected mainly in particulate fractions. The 12,000 x g pellet contained a Ca^{++} sensitive PLA_2 activity, however 10mM Ca^{++} stimulated a two-fold increase, in sharp contrast to the 18-fold increase in PLC activity. PLA_2 activity was also inhibited by MEP, BPB and SEI. We are currently characterizing DAG lipase activity. Our data clearly demonstrated that inhibitors show no specificity for either PLC or PLA_2. The fact that MEP, BPB and SEI blocked the ability of muscarinic agonists to stimulate cyclic GMP formation strongly support the involvement of these phospholipases in receptor mediated events. (Supported by Mayo Fdn. and USPHS Grant MH 27692).

341.15 REDUCTION OF CORTICAL CHOLINE ACETYLTRANSFERASE (CAT) ACTIVITY FOLLOWING INJECTIONS OF ETHYLCHOLINE MUSTARD AZIRIDINIUM ION (AF64A) INTO THE NUCLEUS BASALIS OF MEYNERT (NBM). R. E. Arbogast* and M. R. Kozlowski. Department of Medicinal Sciences, Pfizer Central Research, Groton, CT 06340.

A large portion of the cholinergic innervation of the cortex appears to arise from cell bodies located in the nucleus basalis of Meynert. AF64A, a compound thought to have selectively toxic effects on cholinergic neurons, has been reported to produce a hypofunction of cholinergic terminals in the cortex when injected into the lateral ventricles (Mantione et al., Science 213, 579, 1981). The present study reports the effects of AF64A on one measure of cortical cholinergic functioning, CAT levels, following its injection directly into the nbM.

Male Sprague-Dawley rats were given stereotaxic injections of either 1 or 10 µl of AF64A (0.02 nmoles/µl) or its vehicle (isotonic saline; pH 7.4) unilaterally at two sites along the rostrocaudal axis of the nbM. Cortical levels of CAT were measured 7 to 14 days later as an indicant of the functional level of cholinergic terminals in the cortex. Acetylcholinesterase (AChE) histochemistry was used to examine the cholinergic neurons of the nbM, which stain darkly for AChE.

Injections of 1 µl of AF64A into the nbM produced a noticeable loss of both diffuse AChE staining as well as densely labeled cell bodies in the region of the ipsilateral nbM. These injections also produced an 8% decrease in CAT activity in the central portion of the ipsilateral cortex when compared to the contralateral cortex. Cortical levels of dopamine and serotonin were not affected. Injections of 10 µl of AF64A produced a substantial loss of AChE activity and darkly stained cell bodies in the nbM, with only a small area of non-specific damage adjacent to the cannula tip. In addition, CAT activity was reduced 17% in the ipsilateral cortex. Vehicle injection (1 or 10 µl) had no effect on either AChE staining within the nbM or cortical CAT levels.

These results demonstrate that injections of low concentrations of AF64A directly into the nbM can decrease cortical CAT levels, presumably by destroying the cholinergic nbM neurons while producing little non-specific damage.

341.16 HIGH-AFFINITY BINDING OF [^{3}H]HEMICHOLINIUM-3([^{3}H]HC-3) IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM: A SELECTIVE MARKER FOR HIGH-AFFINITY CHOLINE UPTAKE SITES T.W. Vickroy, W.R. Roeske and H.I. Yamamura. Departments of Pharmacology and Internal Medicine, Univ. of Arizona, Tucson, Arizona 85724.

Among the various elements of the mammalian CNS parenchyma, cholinergic neurons display the unique ability to accumulate choline via a high-affinity transport mechanism. Previous studies have demonstrated that high-affinity choline uptake (HACU) is the primary route by which choline enters precursor pools which are used for transmitter synthesis and that HACU is closely associated with impulse-dependent acetylcholine release. Among the agents that interfere with HACU, hemicholinium-3 (HC-3) is one of the most potent and selective inhibitors known. In this report, we describe the membrane binding properties of [^{3}H]HC-3.

[^{3}H]HC-3 binding was studied in twice washed homogenates of rat CNS tissues. The incubation mixture was prepared in phosphate-buffered medium (pH 7.4) and incubations were carried out for 20 min at 25°C. Membrane-bound and free radioactivity were separated by rapid filtration and specific binding was estimated by adding 1µM unlabelled HC-3.

Preliminary studies indicate that [^{3}H]HC-3 binding is entirely dependent upon the presence of NaCl. Binding increases with NaCl concentrations up to a maximum at or near the NaCl concentration of extracellular fluid. Chloride salts of other monovalent cations and most other sodium salts were much less effective. Direct saturation analysis of [^{3}H]HC-3 binding and indirect competition studies (HC-3 vs. [^{3}H]HC-3) yielded similar estimates for a homogenous population of high-affinity (apparent K_d = 1.9 nM) and saturable (B_{max} = 184±23 fmoles/mg prot.) [^{3}H]HC-3 binding sites in striatal membranes. Studies of the regional distribution of [^{3}H]HC-3 binding sites revealed the following apparent density profile: striatum >> hippocampus > cerebral cortex > cerebellum. In addition, the binding of [^{3}H]HC-3 was relatively insensitive to several metabolic inhibitors but was markedly reduced at lower temperatures.

In conclusion, specific binding of the potent HACU inhibitor [^{3}H]HC-3 has been studied and demonstrates a strong correlation (ionic dependence, apparent affinity, regional distribution, etc.) with previously published data for HACU in rat brain synaptosomes. In view of these and other results, it appears that high-affinity binding of [^{3}H]HC-3 is a selective marker for sites related to HACU and as such represents an important ligand for monitoring this specific functional process of cholinergic nerve terminals.

ACETYLCHOLINE III

342.1 CHOLINERGIC PHARMACOLOGY OF RAT SPINAL DORSAL HORN NEURONS IN VITRO. K. Murase, J. Willetts, L. Urban, and M. Randic. Dept. of Vet. Physiology and Pharmacology, Iowa State University, Ames, IA 50011.

Although the presence of choline acetyltransferase, acetylcholinesterase and muscarinic and nicotinic cholinergic receptors has been demonstrated in the spinal dorsal horn, membrane actions of various cholinomimetic agents and the cholinergic pharmacology of dorsal horn neurons have not been extensively studied.

Responses of dorsal horn neurons to bath application of muscarinic and nicotinic agonists and antagonists as well as ACh were recorded intracellularly utilizing the rat (10-25 days old) spinal cord slice preparation (K. Murase and M. Randic, J. Physiol., 334, 141-153). Membrane resistance was measured either by continuous hyperpolarizing current pulses or by construction of IV plots before, during and after drug application.

ACh ($5x10^{-5}$-10^{-3}M) depolarized (2.4±1.0mV, at 10^{-4}M, mean±S.D., n=13) and increased excitability of about half of the tested cells (n=43). Depolarization was accompanied by a small decrease in membrane input resistance. Nicotine (10^{-6}-10^{-4}M) depolarized (5.3±3.2mV at 10^{-5}M, n=21) 87% of cells (n=37) and also decreased membrane input resistance. While ACh-induced hyperpolarization was observed in a few cells, acetyl-β-methylcholine ($5x10^{-5}$-10^{-4}M) induced hyperpolarization (2.9±1.4mV at $5x10^{-4}$M, n=7), associated with a decrease in input resistance, in 25% of cells (n=36). In addition, acetyl-β-methylcholine induced depolarization (4.0+2.1mV at $5x10^{-4}$M, n=9), associated with increased input resistance in 39% of cells. Carbamyl-β-methylcholine(10^{-4}M) similarly induced both depolarizing and hyperpolarizing responses. Curare (10^{-6}M) and dihydro-β-erythroidine($5x10^{-5}$M) reversibly blocked nicotine-induced depolarization without detectably changing the membrane potential. Atropine(10^{-6}M) abolished the responses to muscarinic agonists and ACh-evoked hyperpolarization. In TTX (10^{-6}M), ACh, nicotine and acetyl-β-methylcholine still induced depolarization with the changes in input resistance described above. In TTX and TEA ($2x10^{-2}$M), ACh and acetyl-β-methylcholine induced depolarizing responses and spontaneous firing of Ca spikes.

The results indicate that ACh-sensitive rat dorsal horn neurons have both nicotinic and muscarinic receptors. The responsiveness of neurons to both nicotinic and muscarinic agonists and the susceptibility of responses to respective antagonists suggests the presence of distinct receptors on individual dorsal horn cells.Support:NIH,Dept. Agriculture.

342.2 DECREASE AND RECOVERY OF ACETYLCHOLINE RELEASE FROM RAT CEREBRAL CORTEX DURING SIMULATED HYPOGLYCEMIA IN VITRO. J.M. Gorell and B. Czarnecki*. Dept. of Neurology, Henry Ford Hospital, Detroit, MI 48202.

To study a possible change in brain acetylcholine (ACh) release during simulated hypoglycemia (HG) in vitro, cerebral cortex (CX) slices from adult male Sprague-Dawley rats were preincubated (10 min) and incubated (15 min; 0.01 µM [^{3}H]choline) at 37°C in Krebs-HCO_3 buffer (K-b) containing 5mM glucose (glc) while gassed (95% O_2:5% CO_2). During HG induction, slices were superfused with gassed K-b containing either control (5mM) or lowered (0.25,0.1,0.05mM)glc, and release of [^{3}H]ACh to 17mM KCl stimulation was tested at 20, 40,60 and 80 min of HG or concurrent 5mM glc exposure. In HG recovery, slices previously preincubated and incubated in 5mM glc K-b were gassed continuously while exposed to either 0.25 or 0.1mM glc K-b for 60 min before a return to 5mM glc K-b in 5.4mM KCl. Recovery of [^{3}H]ACh release to 17mM KCl stimulation was tested at 10,20 and 30 min thereafter, and compared with concurrent, continuously 5mM glc-exposed controls. Fractional rates of [^{3}H]ACh release during 5.4 and 17mM KCl periods were calculated and expressed as a % of concurrent controls (n=3-6/experiment).

There was a [glc]-dependent decrease in [^{3}H]ACh release from CX during HG at 60 and 80 min with 0.25 mM glc (60 min: 71±9%,X±SEM, n=6, p<.02; 80 min:80±4%, n=6, p<.001) and 0.1 mM glc (60 min:56±6%, n=6, p<.001;80min:50±11%, n=3, p<.02). During HG with 0.05 mM glc, there was an earlier (20 min: 80±1%, n=6, p<.001) and greater fall (60 min:40±4%, n=6, p<.001) in [^{3}H]ACh output. During recovery after 60 min of HG, CX exposed to 0.25mM glc increased [^{3}H]ACh release to 87±8% of control (p vs. control N.S.) after 10 min of 5mM glc, but CX exposed to 0.1mM glc reached just 71±4% (p vs. control < .001) of control after 30 min of 5mM glc. There was no difference in spontaneous (5.4mM KCl) [^{3}H]ACh output from0.1 and 5mM glc-exposed CX at 60 min, but the addition of 10 µM 3,4-diaminopyridine (DAP) increased spontaneous [^{3}H]ACh release from these values by 13 (n=3) or 34% (n=3) in 5 or 0.1mM glc (p<.01), respectively. 10 µM DAP's effect in HG CX was maximal, but 5mM glc-treated tissue required 1mM DAP to achieve the same degree of response. These early data suggest that spontaneous ACh release from HG CX is more sensitive to means of augmenting transneuronal Ca^{++} influx to promote ACh release. The decrease in ACh release from HG CX may be a functionally important mechanism in the production of hypoglycemic encephalopathy. (Supported by Fund for Henry Ford Hospital and the Gossett Fund).

342.3 INTRACEREBROVENTRICULAR AF64A ALSO REDUCES ACETYLCHOLINE RELEASE FROM RAT HIPPOCAMPAL SLICES. S.M. Leventer*, D. McKeag*, M.Clancy*, E. Wulfert*, and I.Hanin. Western Psychiatric Institute and Clinic, Dept. of Psychiatry, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15260 and UCB s.a. Pharmaceutical Sector, Brussels, Belgium.

The effect of ethylcholine mustard aziridinium ion(AF64A) on rat central cholinergic function was investigated. Seven or 21 days following bilateral stereotaxic i.c.v. administration of vehicle or AF64A (3 nmoles/3µl/side), striatum, hippocampus and cortex were removed and analyzed for choline acetyltransferase (ChAT) activity, high-affinity choline transport (HAChT) and [^{3}H]QNB binding.

In addition, K^+-stimulated acetylcholine release (KAChR) from superfused hippocampal slices was studied. Following incubation with [^{3}H]choline (0.1µm, 30 min), hippocampal slices (0.5mm thick) were mounted in chambers and superfused with KRB buffer. After 50 minutes of stabilization, the buffer was changed to one containing 40mM KCl (2 x 5 min). This caused a large [^{3}H] efflux, determined by TLC to consist mainly of [^{3}H] ACh. The sum of these two stimulations represented KAChR.

KAChR was reduced to 24% or 35% of control, 7, or 21 days post-AF64A treatment, respectively ($p < .001$). Hippocampal ChAT activity was also reduced, to 58% of control, both 7 and 21 days post-AF64A treatment ($p < .001$); however, striatal and cortical ChAT were unchanged. Hippocampal HAChT was reduced 7 and 21 days post-treatment, to 33.0% ($p < .02$) or 48.0% ($p < .001$) of control, respectively. Hippocampal [^{3}H]QNB binding was unchanged 7 days post-AF64A treatment; however, 21 days post-AF64A treatment, there was a small (11%, $p < .01$) decrease in hippocampal [^{3}H]QNB binding. Neither HAChT nor [^{3}H]QNB binding in striatum and cortex were altered by AF64A treatment.

Under the specific conditions of this study, the effects of AF64A appear to be selective for the hippocampus. In addition to AF64A's reduction of HAChT and ChAT activity, ACh release from hippocampal slices was significantly reduced by AF64A treatment in vivo. These data, using a low dose of i.c.v. AF64A, further emphasize the value of using AF64A as a selective tool for inducing a persistent cholinergic hypofunction in vivo.

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The authors wish to thank Dr. A. Fisher for providing the AF64A used in these studies.

342.4 EFFECT OF dl-2(4-PHENYLPIPERIDINO) CYCLOHEXANOL ON THE SUBCELLULAR STORAGE AND RELEASE OF ACETYLCHOLINE IN MOUSE BRAIN P.T. Carroll Dept. Pharmacol. Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430.

The drug dl - 2(4-phenylpiperidino) cyclohexanol (AH 5183) inhibits (Ki = 40 nM) the active transport of acetylcholine (ACh) into isolated synaptic vesicles prepared from the electric organ of Torpedo californica (Parsons et al., 1984). In the present study, it was of interest to determine whether AH 5183 would be equipotent in reducing the amount of ACh released from brain tissue; also how it might effect such a reduction. The results indicated that a 34 nM conc. of AH 5183 reduced the K^+ induced release of ACh by 56%. It was somewhat less potent in reducing the spontaneous or veratridine-induced release of ACh. Surprisingly, it failed to alter the subcellular levels of ACh during high K^+ depolarization. However, it elevated the level of ACh in the crude vesicular fraction (P_3) fraction during veratridine depolarization by the same percentage as it inhibited the veratridine induced release of ACh. Also, it tended to selectively elevate the level of choline in the P_3 fraction during both high K^+ and veratridine depolarization. AH-5183 inhibited the repletion of the P_3 fraction with newly synthesized ACh more so than the cytoplasmic fraction. Simultaneously, it reduced the amount of extracellular choline available for reuptake. Although AH 5183 reduced the accumulation of extracellular choline by the P_3 fraction at some concentrations (170 nM to 17 uM), it did not do so at 34 nM. Also, it did not inhibit either the soluble or membrane-bound fraction of ChAT at this conc. It is concluded that AH-5183 is a very potent inhibitor of ACh release from brain but its exact mechanism of action in causing this inhibition cannot presently be determined. However, the results obtained in this study tend to suggest that AH-5183 acts on vesicular stores of ACh to reduce the release of ACh from brain tissue. (Supported in part by NSF grant BNS 8117975).

342.5 PURIFICATION OF MEMBRANE BOUND CHOLINE ACETYLTRANSFERASE FROM HUMAN BRAIN. J.H. Peng*, E.G. McGeer and P.L. McGeer (SPON: S.C.Sung). Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Membrane bound choline acetyltransferase (mChAT) was purified from pellet following the extraction from human caudate and putamen of soluble ChAT (sChAT) with 50 mM potassium phosphate buffer, pH 7.4, and centrifugation at high speed. mChAT was solubilized from pellet with 1% Triton X-100 in the same buffer for preparation of sChAT. After centrifugation, mChAT was precipitated with ammonium sulfate at 35-65% saturation. Since mChAT was quite unstable in the presence of protamine sulfate, the crude enzyme was applied directly onto a DEAE-Sepharose column without treatment with this chemical. mChAT was basic and passed through the column unretarded; it was further subjected to hydroxylapatite and phosphocellulose chromatography. Finally, mChAT was applied to a CoA-Sepharose affinity column and was eluted with 1 mM CoA in 10 mM sodium phosphate buffer, pH 7.2 containing 2 mM EDTA, 2 mM β-mercaptoethanol and 10% glycerol. The final preparation of mChAT has a specific activity of 28.5 mol ACh formed per min per mg protein. The purified mChAT migrated as two bands on SDS-polyacrylamide gel electrophoresis with molecular weights of 67,000 and 52,000 daltons, respectively. mChAT has a pH optimum of 8.0-8.3. The K_m(s) for acetyl-CoA and choline were estimated to be about 16.5 µM and 330 µM, respectively.

Immunoblot autoradiography showed that an antiserum prepared previously against sChAT also cross-reacted with both bands of mChAT, indicating that both forms of this enzyme are related. Furthermore, Fab-Sepharose chromatography (Neurochem. Res., 1983) could be used for the purification of mChAT and this preparation also resolved into two bands on our SDS gel. Preparation of a monoclonal antibody to mChAT is in progress.

Supported by MRC of Canada.

342.6 EFFECTS OF LITHIUM AND CHOLINERGIC AGENTS DEPEND ON CHOLINERGIC PROJECTIONS TO HIPPOCAMPUS AND CINGULATE CORTEX M. P. Honchar, M. T. Price, J. W. Olney and W. R. Sherman.* Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110

Systemic administration of LiCl to rats elevates cortical levels of D-myo-inositol-1-phosphate (MIP), which is a product of phosphoinositide metabolism (Sherman, W.R. et al, J. Neurochem. 36: 1947, 1981). We have found lithium (Li) to be a useful tool to identify activity at phosphoinositide-dependent receptors in vivo. Subcutaneous administration of the cholinergic drugs physostigmine or mecamylamine, results in an increase in cortical MIP that is greatly enhanced by Li (Honchar, M.P. et al, Science, 222:323, 1983; Soc. Neurosci. Abst., 9:960, 1983).

All rats received LiCl 3 meq/kg s.c., some animals received either physostigmine (0.4 mg/kg, 1 hr survival) or mecamylamine (50 mg/kg, 2 hr survival). All rats were killed at 25.5 hrs after lithium. In some rats a knife cut in the cingulate radiation and fimbria severed the cholinergic projections to cingulate-parietal cortex and hippocampus. Measurements of MIP levels in those regions were performed 4 days after surgery. The lesion decreased choline acetyltransferase activity by 80% in cortex and by 69% in hippocampus.

In cortex Li produced a 4-fold and Li + physostigmine a 10-fold elevation in MIP above the levels in untreated rats. The effects of both treatments were abolished by the lesion. Li + mecamylamine treatment produced an 8-fold MIP increase that was reduced to 3-fold by the lesion.

In hippocampus the effects of these drugs on MIP levels were of lower magnitude. Nevertheless, the lesions again reduced the elevation of MIP except in the case of Li plus mecamylamine where lesioning produced no change.

These findings support the interpretation that the MIP elevating effects of Li and Li + physostigmine depend on release of ACh from presynaptic terminals. The relative insensitivity to lesioning of the effect of Li + mecamylamine on MIP levels suggests that at least part of the effect of mecamylamine is postsynaptic and independent of the extrinsic cholinergic innervation. It is of interest to note that a single knife cut of the cingulate radiation eliminates the majority of the extrinsic cholinergic innervation of the cingulate-parietal cortex. Supported by USPHS grants NS-05159, AA-03539 and RSA MH-38894 (JWO).

342.7 AFFERENT CONNECTIONS OF NUCLEUS BASALIS IN RAT. J.H. Haring and R.Y. Wang. Departments of Anatomy and Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

Central cholinergic systems arising from neurons of the nucleus basalis (NB) project throughout the neocortex and are thought to participate in a number of functional processes in both the normal and pathologic brain. In particular, the selective deterioration of NB cholinergic neurons is a major consequence of Alzheimer's disease. Although the efferent connections of NB have been the subject of several recent studies, little is known concerning its afferent inputs. We have used horseradish peroxidase (HRP) to identify sources of afferent projections to NB.

Stereotaxic placements of HRP or WGA-HRP were made in NB of Sprague-Dawley rats (225-250g). The enzyme was delivered either by pressure injection (10-20 nl) with a micropipette attached to a Hamilton syringe or by iontophoresis. If the injection was limited to the medial globus pallidus, wherein NB neurons are located, sparse labeling (1-5 cells/nucleus) was seen in the following regions: caudate n, centromedian n, ventromedial n, subthalamic n, zona incerta, parafascicular n, substantia nigra pars compacta, ventral tegmental area, mesencephalic reticular formation, pontine reticular formation pars oralis, parabrachial n, dorsal raphe n and locus ceruleus. In addition, moderate labeling (20-30 cells/section) was seen in frontal cortex corresponding to areas 8 and 10 of Krieger. Cortical neurons labeled from NB appeared to be located mainly in layers IV and V and included both pyramidal and granule cell types.

The present study has demonstrated that NB receives inputs from a variety of regions throughout the neuraxis. Because the rat NB is situated in the medial aspect of the globus pallidus, all subcortical nuclei identified in this study as being afferent to NB also have connections with globus pallidus. Thus the rat NB appears to represent a subset of pallidal neurons with regard to subcortical afferent connections. However, the presence of afferent projections originating in frontal cortex appears to be unique to the medial pallidal region and NB. This observation suggests that the rat NB may be reciprocally related to its cortical targets.

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342.8 REGULATION AND CHARACTERIZATION OF ACETYLCHOLINESTERASE ISOFORMS IN SEPTAL AND HIPPOCAMPAL CULTURES. K. M. Schegg*, K. J. Futamachi* and J. H. Peacock (spon: L. J. Tirri). U. of Nevada School of Medicine, Reno, NV 89557.

Regulation of isoforms of acetylcholinesterase (AChE) has been well described for peripheral cholinergic synapses but not for central neurotransmission. In the mammalian brain, AChE disappears after interruption of the septal cholinergic projection to the hippocampus suggesting that isoforms of AChE may be regulated by septal neurons. This hypothesis can be tested in culture where AChE isoforms may be characterized in hippocampal cultures alone and then compared to those in septal or septohippocampal cocultures, both of which develop cholinergic synapses.

Dissociated septal and/or hippocampal tissue from mice 16-18 days gestation were grown in culture for 7-9 days. AChE was extracted from the cultured cells or tissues taken directly from mice of the same age. The resulting extract was assayed for AChE and centrifuged on isokinetic sucrose gradients. Typical specific activities for plates of medium density were 1-5 μmoles/min per plate; hippocampal activity was generally about half of that of septum alone or septum cultured with hippocampus. AChE was extracted using tris buffers containing high salt plus either 0.5% Triton X-100 or 1% sodium cholate. Both of these buffers extracted nearly 100% AChE from intact tissues but, from cultures, only 84-95% with Triton X-100 and 95-100% with cholate. Sucrose gradients of extracts formed from the 3 types of cultures and from fetal tissues all showed significant peaks at 4.5S and 10S with a shoulder at about 7S. Both septal and septohippocampal extracts yielded AChE profiles with a large 10S peak that constituted about 59% of total activity. Profiles of hippocampal extracts, however, showed only 24% of the activity in the 10S peak. These results were independent of the extraction buffer used and of the presence or absence of horse serum in the medium. Experiments with echothiophate indicated that virtually all 4.5S AChE in septohippocampal and hippocampal cultures was in the interior of the cell while about 2/3 of 10S activity was exterior.

In sum, septum appears to regulate AChE isoform composition in hippocampus; the mechanism of this regulation is currently being investigated.

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342.9 Circadian Variation in the Effects of Imipramine on the Cholinergic Enzyme System of Various Rat Brain Regions. Magdi R. I. Soliman*, Mervin E. Williams*, Timothy Allen* and Charles A. Walker. College of Pharmacy, Florida A&M University, Tallahassee, FL 32307

Imipramine has been shown to alter the circadian rhythm of muscarinic acetylcholine receptors in the rat brain. The present investigation was conducted to study the diurnal variations in the effects of Imipramine on choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities of various rat brain regions. Male Sprague-Dawley rats (150 - 200 g) adapted to a 12 h light:12h dark illumination cycle were used in this study. Imipramine (10 mg/kg) was administered i.p. to rats either at the beginning of the light phase (07:00 h) or at the beginning of the dark phase (19:00 h). Control rats were injected with saline. The animals were sacrificed by decapitation four hours later. The hypothalamus, hippocampus, cerebral cortex and midbrain were dissected and their ChAT and AChE activities were determined by spectrophotometric assays. Imipramine administered at the beginning of the light phase resulted in a significant increase in ChAT activity of the cortex and hypothalamus which was accompanied by a significant increase in AChE activity of the midbrain. No significant changes in ChAT or AChE activity were observed in other brain regions studied. However, when Imipramine was administered at the beginning of the dark phase, it significantly inhibited ChAT activity in the cortex and hypothalamus and significantly decreased AChE activity of the hypothalamus. These results clearly indicate that Imipramine affects the cholinergic enzyme system of specific rat brain regions and that these effects are diurnally controlled (Supported by NASA Grant NSG 2029)

342.10 FUNCTIONAL AND NEUROCHEMICAL CORTICAL CHOLINERGIC IMPAIRMENT FOLLOWING NEUROTOXIC LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS (NBM) IN THE RAT. S.R. El-Defrawy*, F. Coloma*, K. Jhamandas*, R.J. Boegman, R.J. Beninger, and B.A. Wirsching*. Department of Pharmacology and Toxicology, and Department of Psychology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Quinolinic acid (QUIN), an endogenous tryptophan metabolite, injected into the brain produces kainic acid (KA)-like neurotoxic action. We have investigated the effect of QUIN and KA on cortical cholinergic function following stereotaxic injection into the NBM and into the fronto-parietal cortex of rats. The release of ^{3}H-acetylcholine (^{3}H-ACh) from cortical slices, high affinity choline uptake (HACU) and acetylcholinesterase (AChE) activity was measured 7 days following unilateral injections of saline (control), QUIN (60, 150 and 300 nmoles) or KA (4.7 nmoles) into the NBM. These parameters were also examined 7 days after unilateral injections of saline, QUIN (300 nmoles) or KA (9.4 nmoles) into the fronto-parietal cortex.

In animals that received injections of QUIN or KA into the NBM, the potassium-evoked release of ^{3}H-ACh, HACU and AChE was significantly reduced when compared with saline-injected animals. Histological examination of stained brain sections showed a marked loss of cell bodies in the region of the NBM and the globus pallidus. However, despite a large histological lesion, the cortical cholinergic function and biochemical markers were only partially reduced. In animals injected with QUIN or KA into the fronto-parietal cortex, the release of ^{3}H-ACh, HACU and AChE was not significantly affected when compared with controls, although histological examination showed marked loss of cortical neurons.

The results demonstrate that QUIN, an endogenous metabolite, produces a deficit in cortical cholinergic function. The toxic effects of this agent on cortical cholinergic function, as reflected in impaired ACh release, are due to its action on cholinergic cell bodies in the NBM. The cortical slice preparation from QUIN-treated animals, showing impairment of ACh release, may be a useful model for assessing the action of drugs designed to improve cholinergic function.

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342.11 QUINOLINIC ACID NEUROTOXICITY ANTAGONIZED BY KYNEURINIC ACID. R.J. Boegman, K. Jhamandas*, S.R. El-Defrawy*, and R.J. Beninger. Departments of Pharmacology and Toxicology, and Psychology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Quinolinic acid (QUIN), a naturally occurring potent agonist of the N-methyl-D-aspartate type amino acid receptor, causes neuron cell death after application to the striatum or hippocampus. Co-injection of the tryptophan metabolite kyneurinic acid (KYNA) with QUIN (3:1) prevents the development of neuronal lesions [Foster and Schwarcz, Soc. Neurosci. Abst. 9: 1185, 1983]. We have examined the dose-response protective effect of KYNA on QUIN neurotoxicity following injection into the nucleus basalis by measuring cortical ^{3}H-acetylcholine (ACh) release and choline acetyltransferase (CAT) activity.

Rats received a 1 µl unilateral injection of QUIN (60, 90, 120 or 150 nmol) alone or different molar ratios of QUIN to KYNA (1:0, 1:0.5, 1:1, 1:2, 1:3). CAT was measured in cortical homogenates while K (35 mM)-evoked ^{3}H-ACh release was from cortical slices (300 µm) pre-incubated with ^{3}H-choline.

Body weight decreased by 60 g within 3 days of QUIN, but returned to pre-injection values by day 7. Co-injection of KYNA with QUIN prevented the marked decrease in body weight. QUIN alone produced a dose-dependent drop in CAT with a maximal decrease of 53% at 120 nmol. A 50% reduction in ^{3}H-ACh release was obtained at 120 nmol QUIN. Co-injection of QUIN plus KYNA did not produce the neurochemical changes observed with QUIN alone. Thus, a 0.5:1-KYNA:QUIN only gave a 17% decrease in CAT, while 1:1, 2:1 and 3:1 afforded nearly complete protection. In contrast to the decreased release of ^{3}H-ACh from cortical slices observed with QUIN, KYNA with QUIN (3:1) allowed maximal ACh release.

In contrast to Foster and Schwarcz who obtained 78% protection by a 1:1 ratio of QUIN:KYNA, we obtained 83% protection at a 1:0.5 ratio. Our results indicate that, in addition to decreasing CAT, QUIN also causes a parallel reduction in ACh release and that KYNA, a tryptophan metabolite, protects against the neurochemical and functional deficit produced by QUIN.

[Supported by the Ontario Mental Health Foundation.]

342.12 EFFECTS OF SCOPOLAMINE AND NUCLEUS BASALIS LESIONS ON WORKING AND REFERENCE MEMORY IN THE RAT. R.J. Beninger, K. Jhamandas *, R.J. Boegman and S.R. El-Defrawy*. Depts. of Psychology and Pharmacology, Queen's Univ., Kingston, Canada, K7L 3N6.

Working memory refers to recall of recent events of transient importance whereas reference memory refers to information stored over the long term. Accurate performance on a delayed alternation task in a T-maze requires working memory (recall of most recently visited arm) and reference memory (knowledge that food is in arms) whereas accurate performance on a spatial discrimination in a T-maze requires mainly reference memory. To test the hypothesis that cholinergic systems are differentially involved in working and reference memory, the effects of the anticholinergic scopolamine (0, 0.3, 0.6 mg/kg, i.p.) and of unilateral destruction of the corticipetal cholinergic cells of the nucleus basalis (NB) with kainic acid (4.7 nmoles in 1 µl) were evaluated in these tasks. In the alternation task (21 trials per da), rats' (n=15) choice accuracy was decreased by a 30-sec delay between trials ($p<.001$) and by scopolamine ($p<.03$), the higher dose producing the largest impairment ($p<.03$). Spatial discrimination accuracy was not significantly affected by delay or scopolamine. For NB lesioned rats (n=11), acquisition of alternation was impaired ($p<.05$) in comparison to sham lesioned (n=18) and unoperated control rats (n=6). However, all rats eventually reached the criterion of 2 da at 75% or better correct after which a 30-sec delay was inserted before half the trails. Again, there was a delay effect ($p<.001$) and a group by delay interaction ($p<.02$); choice accuracy of the NB group was impaired at the 0-sec delay ($p<.01$) but groups did not differ significantly at the 30 sec delay, possibly because control performance was near chance. Spatial discrimination accuracy was not significantly affected by delay or NB lesions. Assays of cortical choline acetyltransferase (CAT) activity showed a greater than 40% decrease on the NB lesioned side compared to the intact side with no significant change in hippocampal CAT. These results support the hypothesis that cholinergic systems, possibly the basalocortical pathway are importantly involved in working memory but not reference memory. (Supported by OMHF).

342.13 THE RECRUITMENT OF SODIUM-DEPENDENT, HIGH-AFFINITY CHOLINE CARRIERS IN RAT FOREBRAIN SYNAPTOSOMES. R.J.Rylett and E.H.Colhoun*. Department of Pharmacology and Toxicology, University of Western Ontario, London, Ontario, N6A 5C1.

Following increased cholinergic neuronal activity in vivo or depolarization of synaptosomes in vitro there is an increase in the velocity of sodium-dependent, high-affinity choline transport. This effect may depend upon the bioavailability of choline carriers in the terminal membrane but the underlying mechanism(s) has yet to be resolved. In our laboratory, choline mustard aziridinium ion (ChM Az) has proved to be a useful neurochemical probe and ligand for investigations related to the high-affinity transport of choline in synaptosomes. The data reported below shows that ChM Az may provide evidence of additional choline carriers in K^+-depolarized synaptosomes. Rat forebrain synaptosomes were incubated in regular Krebs-Ringer (KR) solution (K^+,5 mM) or high K^+-KR (K^+,40 mM) for 10 min then exposed to ChM Az (0.9 µM) for 10 min before sodium-dependent ^{3}H-choline transport activity was monitored in regular K^+; prior depolarization in control synaptosomes produced a 40% increase in choline transport velocity. In synaptosomes preincubated in regular KR, ChM Az (0.9 uM, 10 min) caused 41% inhibition of choline uptake. This blockade was increased to 58% by prior depolarization (K^+,40 mM) of the nerve ending particles. This could occur if a greater number of carriers were exposed to the outer membrane surface of the synaptosomes per unit time for alkylation by ChM Az. In other aliquots of synaptosomes, the protocol was reversed; that is, synaptosomes were exposed to ChM Az before depolarization. In synaptosomes in which high-affinity choline uptake was blocked by ChM Az, K^+-depolarization produced a stimulation of choline transport. In summary, it would appear that the increased velocity of synaptosomal choline transport induced by depolarization could be mediated by a recruitment of carriers or by movement of carriers from the inside surface of the membrane to the outer surface at a faster rate.

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342.14 SYNTHESIS AND RELEASE OF ^{14}C-ACETYLCHOLINE PRODUCED FROM ^{14}C-GLUCOSE AND INTRATERMINAL CHOLINE POOLS. T.J. Carlton*, E.H. Colhoun* and R.J. Rylett (SPON. M.A. Cook). Department of Pharmacology and Toxicology, University of Western Ontario, London, Ont., Canada, N6A 5C1.

In our laboratory, the efficiency of acetylation of choline following high-affinity transport was markedly different in synaptosomes prepared from rat and guinea-pig forebrain (Rylett, et al., Soc. Neurosci. 9, 971, 1983). The present study was performed to assess the utilization of choline from an intraterminal pool in ACh synthesis using ^{14}C-glucose as a tracer; high-affinity choline transport was blocked by hemicholinium-3 (HC-3) or both high and low-affinity transport were irreversibly blocked by choline mustard aziridinium ion (ChMAz). Following a 30 min incubation with ^{14}C-glucose (70uM) control synaptosomal ^{14}C-ACh values were 314.5±27.3 pmol/mg protein/30 min (x±S.E.M.) for rat and 260.5±34.5 in the guinea-pig. Samples incubated with HC-3 (10uM) or pre-incubated with ChMAz (100 uM for 10 min) showed no significant differences in synaptosomal ^{14}C-ACh content when compared to control values or when compared between species. It was found that intraterminal choline can be used for the synthesis of ACh to a similar extent under conditions where choline transport has been blocked by known inhibitors of the carrier mechanism. Subcellular fractionation studies were performed to compare cytoplasmic versus occluded incorporation of ^{14}C-ACh between the species. In rat brain synaptosomes 68.2±2.21% of the ^{14}C-ACh synthesized was recovered in the cytoplasmic fraction (S_3) compared to 61.0±1.95% in the S_3 fraction from guinea-pig synaptosomes. The amount of vesicular incorporation in the rat and guinea-pig when comparing controls to HC-3 or ChMAz-treated samples did not differ significantly between the species nor between the treatment groups. Analysis of spontaneously released ^{14}C-ACh in control samples showed values of 0.64±0.06 pmol/mg protein/min from rat synaptosomes compared to 0.20±0.03 for the guinea-pig ($p<0.005$). Spontaneous release of ^{14}C-ACh was significantly reduced by 85.5% in HC-3 samples and by 82.7% in ChMAz samples in the rat ($p<0.005$). In the guinea-pig the corresponding reductions were 56.6% and 63.3% respectively ($p<0.005$). Decreased spontaneous release of ^{14}C-ACh release in HC-3 or ChMAz-treated samples was not due to a difference in subcellular distribution of newly-synthesized ^{14}C-ACh, but indicates that inhibitors of choline transport can inhibit spontaneous release in the absence of intracellular ^{14}C-ACh changes.

(Supported by the Medical Research Council of Canada).

342.15 THE RELATIVE SELECTIVITY OF ANTICHOLINERGIC ANTIPARKINSONIAN DRUGS FOR M1 AND M2 MUSCARINIC RECEPTOR SUBTYPES.
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The receptor binding of classic muscarinic antagonists is adequately described by a one-site model, but the binding of the novel antagonist, pirenzepine, is not. Its binding properties support the concept of muscarinic receptor subtypes, M1 and M2. Autoradiographic studies have shown that forebrain muscarinic receptors are predominantly M1, whereas hindbrain receptors are predominantly M2 (Mash, Potter, 1982; Wamsley et al 1984).

While the anticholinergic antiparkinsonian drugs are recognized to differ in potency, the possibility that they may also differ in their selectivity for the muscarinic subtypes has not been studied in detail. We have examined the selectivity of these drugs by studying their ability to displace ^{3}H(-)QNB from rat forebrain (predominantly M1) and hindbrain (predominantly M2) membrane preparations. Membranes (26.0 ± .5 μ protein/4.0 ml incubate) and ^{3}H(-) QNB (120 pM) were incubated (37°C, 60 minutes in 50mM $NaKPO_4$, pH 7.4) in the presence of varying concentrations of drugs. The Ki for each drug for forebrain and hindbrain membranes was calculated from IC50 's using the Cheng and Prusoff equation and experimentally determined K_D's for ^{3}H(-) QNB in forebrain (K_D= 22 pM) and hindbrain (K_D= 28 pM). We expressed selectivity for M1 as a ratio of Ki hindbrain/Ki forebrain. As expected, pirenzepine had the greatest seletivity with a ratio of 8.6. All drugs studied had a higher affinity for forebrain receptors; thus the lowest selectivity ratio was 2.1 (orphenadrine).

We found that these drugs varied up to 3-fold in selectivity. In descending order of selectivity (drug, KiH/KiF): biperiden (Akineton), 6.8; trihexyphenidyl (Artane), 6.4; scopolamine, 5.5; dexetimide (Tremblex), 4.3; benztropine (Cogentin), 4.2; atropine, 3.3; procyclidine (Kemadrin), 2.9; ethopropazine (Parsidol), 2.4; orphenadrine (Disipal), 2.1.

The varying selectivity among these drugs indicates they may differ in therapeutic index, depending on whether benefit is mediated at M1 or M2. The development of even more selective drugs will not only permit more selective treatment, but will provide information about the brain region where benefit is mediated.

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342.16 IN VIVO AND IN VITRO EFFECT OF TETRACAINE ON MEMBRANE-BOUND ACETYLCHOLINESTERASE ACTIVITY.
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Intraperitoneal administration of a single dose of tetracaine(25 mg/kg,ip) to rats produces a time dependent(30-240 min) significant stimulation of acetylcholinesterase(AChE) activity(20.7-40.0%) in brain synaptosome. Erythrocyte membrane-bound AChE activity, on the other hand, is significantly inhibited(20.8-34.2%) under similar conditions of treatment. Tetracaine, under in vitro condition, produces a significant and concentration dependent reversible inhibition of AChE activity in synaptosome(4.4-100%) and erythrocyte membrane(16.0-100%) at concentrations 25-300 μM and 50-400 μM respectively. The IC50 values of tetracaine in synaptosomal and erythrocyte membrane-bound AChE are 88 and 200 μM respectively. Tetracaine at sub-inhibitory concentrations(10 μM) produces a small but significant stimulation(8.7-23.0%) of AChE activity in synaptosome but not in erythrocyte membrane. Lineweaver-Burk plots indicate that the Km(2.5mM) of synaptosomal AChE is increased(100-500%) with the increase of tetracaine concentrations(50-150 μM) and likewise the Km of erythrocyte membrane (0.68mM) is increased(124.5-546.9%) with the increase of drug concentrations(100-300 μM). In both the membranes Vmax of AChE remains unaltered under similar condition of treatment with tetracaine.

Present results suggest that tetracaine, depending on its concentration, produces a biphasic action on AChE in neuronal membrane but not in non neuronal membrane.

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NEUROTOXICITY II

343.1 THE RELATIVE POTENCY OF ALKALOID ANTIMITOTIC DRUGS AS INHIBITORS OF GOLDFISH OPTIC NERVE REGENERATION IN VIVO.
R.E.Davis, B.E. Schlumpf and P.D. Klinger. Univ. of Michigan, Ann Arbor, MI 48109.

Experiments were carried out to investigate the regenerating goldfish optic nerve as a preparation for screening drugs for adverse effects on neuronal circuit development. Regeneration was induced by unilateral retrobulbar optic nerve crush and the opposite eye was kept intact. The time to recovery of vision was measured, as an index of regeneration and neurotoxicity, by a classical conditioning method that permits sequential and monocular testing within individuals. The CS consisted of the up-down movement of a spot of red light, the US was an electrical body-shock, and the response index of conditioning was the occurrence of a branchial suppression response (SR) during the CS-US interval. The moving-spot SR is mediated by visual input to the optic tectum (Schlumpf, B.E. and Davis, R.E. Neurosci. Abstr. 10, 1984). Optic nerve crush fish showed visual recovery at between 14 to 25 days postaxotomy (30°C). This is similar to the time interval required for extensive axonal ingrowth to the contralateral optic tectum (Springer, A.D. & Agranoff, B.W., Brain Res., 128:405, 1977). The alkaloids, colchicine, podophyllotoxin (Sigma), vinblastine sulfate and vincristine sulfate (Lilly), which are varyingly potent as inhibitors of mitosis, microtubules and axonal transport (Hanson, M. & Edstrom, A., Int. Rev. Cytol. Suppl., 7:373, 1978) were administered semiweekly by ip injection beginning the day prior to optic nerve crush. Each inhibited recovery of response with the experimental eye at doses that did not inhibit maintenance of response with the control eye. The 50% effective dose (and 95% confidence limits) in decreasing order of potency was vincristine, 0.02 (0.004 to 0.034) μg/g body wt., podophyllotoxin, 0.06 (0.03 to 0.17) μg/g, colchicine, 0.08 (0.06 to 0.1) μg/g, and vinblastine, 0.53 (0.18 to 1.0) μg/g. Lumicolchicine (Sigma) or picropodophyllotoxin (Wilson, L. & Friedkin, M., Biochemistry, 5: 2463, 1966), which are much less potent inhibitors of microtubules or axonal transport than their isomers, had no effect on regeneration. Thus, inhibition of axonal transport or microtubules could be responsible for the inhibition by systemic colchicine or podophyllotoxin of optic nerve regeneration. These experiments show that the behavioral assay of optic nerve regeneration can screen molecules that may impair neuronal circuit development in vivo and measure their relative potency.

343.2 IN VIVO LEAD TREATMENT INCREASES THE BINDING OF CALCIUM ANTAGONISTS BINDING TO RAT STRIATAL MEMBRANES - S. Govoni, A.Rius* L. Lucchi*, F. Battaini, M. Trabucchi[a]. Institute of Pharmacology and Pharmacognosy, University of Milan and [a]Chair of Toxicology, 2nd University of Rome, Italy.

Lead intoxication modifies various neurochemical parameters; the effect of lead on neurotransmission may be at least partially due to an interference with the availability of calcium for neurotransmitter release. In vitro data on striatal synaptosomes indicate that lead potentiates calcium dependent dopamine release. In addition, lead may decrease the affinity of mitochondria for calcium leading to an intracellular accumulation of this ion, possibly harmful for the neuron itself. On this line we investigated whether chronic in vivo lead exposure alters the binding characteristics of ^{3}H-Nitrendipine (^{3}H--NDP), a ligand used for studying calcium channels in different tissues including the brain. Pregnant Sprague-Dawley rats were used. Animals at day 16 of pregnancy were given lead acetate (1360 ppm) in their drinking water. Three weeks after birth the young rats were separated from the mothers and divided by sex. They continued on the same drinking solution which had been supplied to their mothers. At 6-8 weeks of age animals were killed by decapitation, striatal tissue dissected and used for binding studies according to Gould, Murphy and Snyder (P.N.A.S., 79, 3656,1982). In control tissue the binding of ^{3}H-NDP was dependent on the presence of calcium ions. In vitro, lead shared the action of calcium in enhancing ^{3}H-NDP binding to crude synaptic membrane preparations, although it was more potent on a molar basis. In vivo lead exposure enhanced ^{3}H-NDP binding to striatal synaptic membrane preparations (from 93±13 to 138±14 fmoles/mg protein; K_D were unmodified). This effect was lost when membranes were washed with EDTA-EGTA (10 μM each) indicating that the increased binding was probably due to the persistence of lead in the synaptic membranes of treated rats. The present results strenghten the concept of an interaction of lead and calcium at neuronal level at membrane sites regulating calcium entry. The observed alterations of ^{3}H-NDP binding may be relevant to the changes in neurotransmitter function observed at striatal level following lead exposure. (Supported by CNR contract 830297656).

343.3 DSP4 REDUCES ENDOGENOUS NORADRENALINE IN SLICES OF RAT CEREBRAL CORTEX BY STIMULATING ITS RELEASE. M. E. Landa*, M.C. Rubio* and G. Jaim-Etcheverry. Inst. Investigaciones Farmacológicas, CONICET and Inst. Biología Celular, Fac. de Medicina, 1121 Buenos Aires, Argentina.

DSP4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine) is a neurotoxic compound that blocks noradrenaline (NA) uptake and depletes endogenous NA in the CNS and in the periphery. The alkylating compound produces these effects after systemic injection (Jaim-Etcheverry, G. and Zieher, L.M., Brain Res., 188: 513, 1980; Jonsson, G. et al., Eur. J. Pharmacol., 72: 173, 1981). In order to investigate the mechanism of the NA depleting action of DSP4, slices from the cerebral cortex of rats, were incubated in the presence of the compound. When DSP4 was present in the incubation medium at a concentration of 10^{-5} M for 60 min, cortical NA was depleted by 40 %. This effect was blocked by 10^{-5} M desipramine.

The depleting action of DSP4 on endogenous NA in the cortex could be due to an alteration of the synthesis or metabolism of NA or to stimulation of its release. To investigate the latter possibility, slices labeled in vitro with ^{3}H-NA (4×10^{-7} M) were incubated in the presence of 10^{-5} M DSP4 for 60 min. In this condition, the spontaneous outflow of radioactivity was markedly enhanced during exposure to DSP4 as well as during the subsequent washings. The radioactivity released by DSP4 was essentially accounted for by NA and 3,4-dihydroxyphenylglycol (DOPEG). The release of radioactivity was independent of the concentration of Ca^{++} in the medium. Since the release of radioactivity was not observed after endogenous NA was depleted by reserpine pretreatment of the rats, that radioactivity apparently originates from the vesicular pool.

Thus, NA depletion caused by DSP4 seems to be due to an enhancement of its release from the vesicular pool. This is further supported by the lack of effect of DSP4 on the en enzymes related to NA synthesis. Only monoamine oxidase activity was reduced by high concentrations of DSP4. The results obtained also indicate that fixation of DSP4 to the NA transport system is necessary but not sufficient to prod produce the acute NA depletion and the characteristic long-term actions of the compound.

(Supported by CONICET and SUBCYT, Argentina)

343.4 ACUTE PHYSIOLOGICAL RESPONSES OF MOLLUSCAN NEURONS TO COPPER. W.F. Wonderlin and D. Weinreich, Dept. of Pharmacology & Exp. Therapeutics, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

During preliminary investigation of copper's effects on chemically-gated ionic conductances in identified neurons of Aplysia californica, we observed that ionic copper (1-100 μM) consistently depolarized the neuronal membrane potential and occasionally produced cell death. We subsequently investigated the basis of these effects by studying the responses of Aplysia neurons to copper salts ($CuCl_2$ and $CuSO_4$) under current-clamped and voltage-clamped conditions using standard intracellular microelectrode techniques.

External application of copper, 1 μM and greater, produced a rapid and reversible membrane depolarization, a decreased input resistance (RI), and an increased slope of the current/voltage (I/V) curve (n=71). The time course of these effects was paralleled by an increased inward Na^+ current. The copper-activated Na^+ current was restricted to the cell soma, and was not observed with intracellular recordings from axons isolated from their cell soma. Intracellular somatic injection of copper, by contrast, produced a hyperpolarization, an increase in RI, and a decreased slope of the I/V curve. This differential effect of copper indicates that the copper-activated Na^+ current results from copper's interaction with a target site located on the external surface of the somal membrane.

The copper-activated Na^+ current was not antagonized by tetrodotoxin (100 μM), procaine (1 mM), $NiCl_2$ (5 mM), curare (1 mM), hexamethonium (1 mM), strychnine (500 μM), dithiothreitol (100 μM), or superoxide dismutase (100 μg/ml). The copper-activated Na^+ current was partially blocked (60-70%) by lidocaine (1 mM) and high-Ca^+ (55 mM) sea water. The copper-activated Na^+ current cannot be accounted for by: (1) interaction of copper with known voltage- or chemically-gated ionic conductances; (2) inhibition of the Na/K-ATPase; or (3) generalized increases in membrane permeability resulting from copper-induced lipid peroxidation. These results support the conclusion that copper activates a unique Na^+ conductance in molluscan neurons.

343.5 DOXORUBICIN, A SUICIDE TRANSPORT AGENT IN THE PERFORANT PATH SYSTEM. G. Bing* and P.D. Coleman. Department of Anatomy, University of Rochester, Rochester, NY 14642.

Suicide transport is a technique being developed to produce highly selective neuronal loss in specific sites of the nervous system. The method involves delivering a cytotoxic agent to the axon terminals of a neuronal population. The cytotoxic agent is taken up by the terminals and transported back to the cell body where it causes death of the neuron (Wiley et al., 1982). Doxorubicin is one such agent. To characterize its neurotoxicity in CNS suicide transport doxorubicin was studied in the perforant pathway, i.e., retrograde transport from the outer two-thirds of the molecular layer of the dentate gyrus to layer II neurons of lateral entorhinal cortex.

A total of 44 male F344 rat brains were examined at 1 hr., 2 hr., 4 hr., 1 day, 2 days, 4 days, 7 days, 11 days, 20 days, 30 days, 3 months, and 6 months after injection of 100 nl of 10% doxorubicin into the molecular layer of the dorsal blade of the dentate gyrus. Animals were sacrificed by injection with an overdose of nembutal i.p. and perfusion with 10% neutral formalin. After cryoprotection with 30% sucrose, frozen sections were cut at 20 um. Sections were counterstained with 0.00001% nuclear yellow.

As early as 1 hour following injection, the cytoplasm of some neurons in the ipsilateral layer II of lateral entorhinal cortex were labeled with the brilliant orange color of doxorubicin fluorescence. Nuclei were labeled with fluorescence by 24 hours. One week after doxorubicin injection, the orange nuclear labeling in layer II appeared scattered and diffuse and some glial cells were labeled. At 20-30 days following doxorubicin injection loss of many layer II neurons of entorhinal cortex could be seen in Nissl stained sections. At this time doxorubicin could be seen in the walls of blood vessels. By 3 months most neurons (up to 70%) in layer II of lateral entorhinal cortex were lost as revealed by Nissl stain. Some layer II neurons were still labeled by a fluorescence that resembled that of doxorubicin.

It is concluded that doxorubicin is a useful agent for suicide transport. Its neurotoxic action could be a useful tool to selectively kill a specific group of neurons in the CNS. In addition, its fluorescent property and rapid axonal transport may make doxorubicin a useful agent for retrograde transport in neuroanatomical studies. Supported by grant AG 1121 from the National Institute on Aging.

343.6 EFFECT OF ORGANOPHOSPHATES ON MUSCARINIC RECEPTOR BINDING IN PC12 CELLS. G.B. Viana*, L.H. Davis*, and F.C. Kauffman. Dept. Pharmacology & Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD 21201.

Rat pheochromocytoma PC12 cells were used as a model to explore mechanisms associated with decreases in muscarinic receptor binding produced by organophosphates. Exposure of native cells to 50μM soman or sarin for 24 hr caused a 40-60% decrease in N-[^{3}H]-methylscopolamine ([^{3}H]-NMS) binding measured in intact cells. A similar decrease was noted in cells exposed to the carbamate, pyridostigmine (100 μM) or DFP (500 μM); however, two other organophosphates, tabun, and VX lowered binding only 5-20%. Transformation of the cells by prior exposure to NGF (50 ng 7S/ml) for 3 days increased binding and potentiated the effects of organophosphates.

	Soman Exposure	N-[^{3}H]NMS Binding fmol/mg protein	molecules/cell
Control	0	31.8	3040
	1 hr	27.3	2340
	24 hr	18.9	2140
NGF	0	39.8	5480
	1 hr	29.5	2740
	24 hr	19.2	2220

In contrast to results with native cells, tabun and VX decreased muscarinic receptor binding to the same extent as soman and sarin in NGF-treated cells. The four organophosphates tested caused significant decreases in cholinesterase activity; however, changes in muscarinic binding produced by these compounds do not appear to occur secondarily to changes in acetylcholine. Carbachol (100 μM) caused a 50-60% decrease in [^{3}H]-NMS binding in 10 min and this decrease was antagonized by atropine (1 μM). Decreases in muscarinic ligand binding produced by organophosphates were much slower (hours) and not antagonized by atropine. Thus, decreases in muscarinic receptor binding induced by organophosphates do not occur via rapid receptor desensitization mechanisms. Receptors remaining after organophosphate treatment differed in both affinity and number. Carbachol displacement curves indicated that IC50 values were significantly lower in cells exposed to organophosphates, but were comparable in native and NGF-transformed cells. Decreases in muscarinic receptor binding produced by organophosphates are relatively specific since these compounds did not alter the specific activities of a number of intracellular enzymes. Supported in part by USAMRDC Contract No. DAMD-17-81-C-1279 and Brazilian National Research Council (CNPq).

343.7 **PROGRESSIVE HIPPOCAMPAL ELECTRICAL CHANGES FOLLOWING MULTIPLE INFUSION OF ALUMINUM CHLORIDE.** Michael Donadio,* Moon He Lee, Laura Scott* and Henryk Wisniewski*; New York State Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y. 10314

An elevated aluminum content in the brain is believed to be neurotoxic and has been claimed to be related with certain CNS degenerative conditions including senile and presenile dementia of the Alzheimer type. In experimental animals (rabbits, cats, ferrets) it has been demonstrated that subarachnoid injection of aluminum salts results in progressive encephalopathy associated with neurofibrillary degeneration, similar but not identical to those seen in humans. In addition, animals have also shown learning deficits. The specific role of aluminum in the functional impairments, however, could not be established with this animal model, because it produces not only widespread neuropathological changes but other neurological symptoms as well. In the present study, using a repeated slow infusion procedure, we attempted to localize the aluminum effects in the brain. Adult male ferrets were chronically implanted with 10 cortical and two pairs of bilateral hippocampal depth electrodes surrounding an injection cannula. After a baseline EEG recording and control infusion with ringers, one of the hippocampi received twice a week a 5ul infusion of 1% $AlCl_3$ (1ul/min) for 3 weeks. The other side was given the same volume of pH-adjusted ringers. Changes in the brain electrical activity in the aluminum infused hippocampus included: (1) Immediate reduction in the amplitude of the hippocampal theta activity; (2) Loss of hippocampal theta activity. Further aluminum treatment results in electrical activity characterized by occasional spikes or spike and slow waves, and in some cases low voltage fast waves in the late stage of encephalopathy, (3) Appearance of high amplitude spikes (up to 1000 uV) and epileptiform discharges, and (4) Cortical EEG showing a decreased amount of fast waves, while slow waves increased. The ringers infused side, on the other hand, showed no distinctive changes until the very late stage, and often the theta activity was preserved until the animals were sacrificed or died. Neuropathological examination revealed no massive neurofibrillary changes at the injection site or in the cerebral cortex. The data suggest that gradual accumulation of aluminum results in progressive changes in the brain electrical activity. The implication of the findings will be discussed in relation to the functional changes reported with Alzheimer's patients.

343.8 **REGIONAL 3H-SPIPERONE BINDING IN RESPONSE TO SUB-ACUTE SOMAN INTOXICATION.** J.J. Valdes, N.A. Chester* and T.-M. Shih. CRDC and USAMRICD, Aberdeen Proving Ground., MD. 21010.

Sub-acute exposure to anti-cholinesterase compounds induces, in humans, cognitive and emotional deficits which include memory loss, withdrawal, depression, anxiety and emotional lability. Neuroleptic drugs used to treat similar clinical conditions bind to serotonergic (S2) and dopaminergic (D2) receptors. It is therefore likely that these "neuroleptic" receptors mediate the cognitive and emotional consequences of sub-acute anti-cholinesterase intoxication. Serotonergic neurons in the raphe nuclei regulate cholinergic systems in the hippocampus (HIP), striatum (STR) and cortex (COR) , and the dopaminergic nigro-striatal pathway makes synaptic contact with STR cholinergic interneurons. 3H-spiperone binds to both S2 and D2 receptors in the COR and STR , respectively, and is an ideal ligand with which to probe the neuroleptic receptors in these systems. To assess post-synaptic response to cholinergic intoxication, rats were administered soman (50 ug/kg, s.c.) three times weekly for four weeks. Synaptic membranes were prepared from HIP, STR and COR from rats killed 1 hr, 1 day and 3 days after the final soman injection. Kinetics of spiperone binding ($7-200 \times 10^{-11}$ M) was assessed using 10 uM either mianserine or haloperidol to identify binding to serotonergic (S2 - HIP & COR) or dopaminergic (D2 - STR) receptors. In some instances, both displacers were added together to exclude the possibility that both receptors were being assessed. Scatchard analysis provided graphical representation and kinetic constants were determined by computer analysis. Analysis of COR binding revealed a non-linear plot indicative of two populations of binding sites. Soman treatment increased Kd of the high affinity component at 1 hr after the final injection with a return to normal by 1 day. Binding in HIP was linear, indicative of a single population of sites which soman reduced by approximately 50%. This reduction in number of binding sites was still evident on day 3, the last day of the study. Binding in STR was non-linear and indicated two populations of binding sites. Soman increased the Kd of the high affinity component and this change was still evident by day 3. The loss of HIP receptors and the decreased affinity of surviving COR and STR receptors suggests that pharmacologic manipulation of these systems with neuroleptic drugs might modulate the sub-acute toxicity of soman.

343.9 TREMORGENIC MYCOTOXINS ALTER SYNAPTIC TRANSPORT OF GABA AND GLUTAMATE. N.A. Chester*, J.J. Valdes, and R.J. Cole* (SPON: H. Popolow). CRDC, TOX BR, APG, MD 21020, and U.S. Dept. Agriculture, Dawson, GA 31742.

Symptoms of mycotoxicosis in both humans and cattle often include tremors and seizures. These neurologic manifestations result from ingestion of tremorgenic mycotoxins derived from common fungi. The neurotoxic mechanisms of these potent tremorgens are obscure, but are thought to involve perturbation of synaptic transport of amino acid neurotransmitters. We therefore evaluated the consequences of intoxication for brain regions associated with different aspects of tremorgenic symptomology. Synaptosomes were prepared from regional brain tissue of albino rats treated with a single dose of dimethylsulfoxide vehicle or a tremorgenic dose of aflatrem (3 mg/kg i.p.) or verruculogen (1 mg/kg i.p.). In vitro toxin effects were assessed by incubating intact synaptosomes with the vehicle or tremorgens. Uptake , spontaneous efflux, and K+-stimulated release of tritiated gamma-aminobutyric acid or glutamate were assessed in hippocampal (HIP), striatal (STR), and cortical (COR) synaptosomes. Verruculogen and aflatrem exert numerous effects on synaptic uptake and release which depend on brain region and route of administration. The most consistent effect was decreased uptake of both transmitters following in vitro toxin exposure, with aflatrem being a more potent inhibitor and the HIP being most sensitive. The in vivo effects were smaller but consistent with these results. COR and STR release mechanisms were reciprocally responsive to both verruculogen and aflatrem, with COR and HIP showing depression, and STR elevation, of stimulated release. These data indicate that tremorgens exert their effects at the synaptic level via modulation of amino acid neurotransmitters, and extend previous reports of differential sensitivities of cortical and subcortical structures.

343.10 IN VIVO AND IN VITRO RESISTANCE TO GLUTAMATE AND KAINATE IN A POPULATION OF LUCIFER YELLOW LABELED RETINAL NEURONS FROM CHICK EMBRYOS. G.D. Zeevalk and A.G. Hyndman, Rutgers Univ., Department of Biological Sciences, Piscataway, N.J. 08854.

Lucifer yellow is known to label a distinct population of cells in the developing chick retina at 11 days in vivo. These cells are found in the innermost part of the inner nuclear layer (amacrine cells) and as a single layer of cells in the ganglion cell layer (displaced amacrines). Cells within these regions are the targets for glutamate and kainate toxicity. We have found, however, that most lucifer yellow positive cells in the retina are not lost following in vivo exposure to glutamate or kainate. Eleven and 12 day chick embryos were given intraocular injections of either 100 nmoles kainate or 1 µmole glutamate and allowed to develop another 20 hours before lucifer yellow labeling. Histologically, the injected eyes showed swelling of cells in the inner part of the inner nuclear layer, extensive swelling and vacuolization of the inner plexiform layer and swelling of some of the cells in the ganglion cell layer. In contrast, lucifer yellow fluorescence of toxin treated retinas was similar to controls although the density of labeling in certain regions was slightly reduced and there was loss of label from some displaced amacrines. In ovo injections of 10 µmoles kainate or 167 mmoles glutamate and reincubation for 48 hours or longer, as well as the in vitro incubation of whole eyes in 5 mM glutamate or 0.2 mM kainate gave similar results.

In vitro, lucifer yellow labeled approximately 2-3% of retinal neurons from 11 day embryos and did not change when labeled at either 24, 48 or 72 hours. This is the same percent of the population that is labeled in vivo. When cultured retinal neurons were treated with either 5 mM glutamate or 0.2 mM kainate at 48 hours in vitro and then incubated an additional 24 hours before labeling, the number of neurons taking up lucifer yellow was not changed appreciably from controls even though there was a 50% decrease in the total number of neurons surviving toxin treatment. This is consistent with the toxin resistance of the lucifer yellow positive cells seen in vivo. Studies to correlate the fluorescent population in vitro with those in vivo are being performed. Also being investigated is the high affinity uptake profile of these lucifer yellow labeled, toxin-resistant cells which should further our understanding of the mechanisms underlying the resistance of these neurons to the toxins.

343.11 DIFFERENTIAL EFFECTS OF AGE OF ADMINISTRATION ON MSG-INDUCED REPRODUCTIVE DISTURBANCES IN MALE AND FEMALE MICE.
June E. Barnhart* and William J. Pizzi. Neuropsychology Lab Northeastern Illinois University, Chicago, IL 60625.

Neonatal administration of monosodium glutamate (MSG) has been shown to produce lesions in the brains of various mammals, with neuronal damage most often being reported to occur in the arcuate nucleus of the hypothalamus. Concomitant with this CNS damage, there have been numerous reports of somatic and behavioral deficits that become manifest in adulthood, including obesity, stunted body lengths, reproductive dysfunction, abnormal activity levels, and reduced seizure thresholds. Furthermore, reduced weights of endocrine glands have been reported in rodents of both sexes, suggesting that neonatal administration of MSG results in an impairment of hypothalamic-hypophysial regulation.

It has also been demonstrated that when mice are treated later in the course of development these animals show mixed effects, with somatic and behavioral abnormalities taking longer to occur or not occurring at all. This suggests that the MSG-induced changes may be an age dependent phenomenon linked to the development of the blood-brain-barrier.

The present report examines the reproductive capacity of male and female mice treated with MSG as neonates (days 2 through 11) or as juveniles (days 25 through 34). Neonates of both sexes consistently demonstrated reproductive dysfunction; however, only male mice treated with MSG as juveniles developed reproductive deficits. Neonatal treatment with MSG resulted in significantly lowered weights of pituitary and thyroid of both sexes, as well as testes or ovaries. Several studies in our lab show that in juvenile mice treated with MSG, only the males showed a reproductive or behavioral deficit with a concomitant reduction in the weights of the pituitary, thyroid, and testes. These results suggest that development of the blood-brain-barrier offers only limited protection against the excitotoxic amino acids. The possibility that males may be more sensitive than females at this age deserves further investigation.

(Supported by a grant from the Committee on Organized Research, Northeastern Illinois University)

NEUROTOXICITY III

344.1 POSTNATAL DENDRITIC AND SYNAPTIC MATURATION IN RATS EXPOSED TO HALOTHANE IN UTERO.
E. Uemura, W. P. Ireland, E. D. Levin, and R. E. Bowman. Dept. of Vet. Anat., Iowa State University, Ames, IA 50011, and Dept. of Psych., University of Wisconsin, Madison, WI.

Dendritic branches and synapses were quantitated in 128 rats at 5, 21, 34 and 95 postnatal days in the entorhinal cortex and subiculum. These rats were offspring of mothers that had been subjected to four different levels of halothane while they were gestating. The exposure conditions were control, intermittent halothane (25 ppm or 100 ppm halothane, 8 hours/day, 5 days/week) and continuous halothane (25 ppm halothane, 24 hours/day, 7 days/week). The control group had longer and more dendritic branches than any of the halothane exposed groups ($p < 0.001$). Dendritic growth in terms of branch numbers and length, was most advanced in the control groups, followed by those groups exposed to 25 ppm halothane intermittent, 25 ppm continuous and 100 ppm halothane intermittent ($p < 0.001$). The latter two exposure conditions exerted identical effects on dendritic growth and syanptic population. The order of this dendritic growth level established at 5 postnatal days remained the same throughout the first 95 postnatal days in both the cortex and subiculum. Thus, the delay in the initial dendritic growth caused by halothane was not compensated by increased rate of growth in the treated groups at later ages. Delayed synaptogenesis caused by halothane was also detected by the electron microscopic observation of growth cones. Growth cones were detected in halothane exposed rats up to 34 days as compared with 21 days in the control. The synaptic density in halothane-exposed rats did not reach the control level even at 95 days, suggesting the enduring effects of halothane on the early synaptogenesis and dendritic growth.

344.2 AGE VARIATION IN RAT BRAIN CORTICAL NA,K-ATPASE ACTIVITY AND THE INHIBITORY ACTIONS OF ERYTHROSIN B.
S.M. Anderson. Neurotoxicology Section, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205.

Na,K-ATPase in parietal cortex from brains of male genetically heterogeneous (NIH/N) rats was studied. Two age ranges were examined: the neonatal development stages (5, 10, 15, 20, and 30 days old) and adult maturation (30, 60, 90, 120, and 240 days old). Measurements of Na,K-ATPase catalytic activity indicate that there is a five-fold increase in enzyme activity between five and twenty days of age. The same level of Na,K-ATPase activity is found in the parietal cortical membrane preparations from twenty, thirty, and sixty day old animals. A small increase in enzyme activity is found in similar preparations from 120 day old animals but significantly lower enzyme activity is found in brain preparations from animals greater than 240 days old (28 percent lower than 30 and 60 day old levels).

A classical pattern of ligand-receptor binding kinetics is not apparent in data from our ouabain binding studies on cortical membrane preparations of five day-old rats. Saturation isotherms and Augustinsson plots of [^{3}H]ouabain (0.25 - 300 nM) binding to crude synaptic tissue preparations from parietal cortex of rats 10 days of age suggest only one ouabain binding site; whereas two ouabain binding sites are demonstrable in tissue preparations from more mature animals. A comparison of Arrhenius plots of temperature-enzyme activity data obtained from 10 and 30 day old animals suggest differential phospholipid content in parietal cortical membranes during postnatal development. Despite these apparent structural differences in synaptic membrane Na,K-ATPase from parietal cortex between 10 and 30 day-old rats; preliminary data suggest a similar pattern of inhibition of Na,K-ATPase catalytic activity and [^{3}H]ouabain binding by erythrosin B (U.S.F.D.&C. Red #3).

344.3 CHOROID PLEXUS IN MAN: A PROTECTIVE SINK FOR HEAVY METALS? L. A. Hershey, C. O. Hershey*, A. W. Varnes* and T. Wongmongkolrit*. Depts. of Neurology, Medicine and Pathology, Case Western Reserve Univ. Sch. of Med. & SOHIO Research & Development Center, Cleveland, Ohio 44106

In human choroid plexus, lead concentrations have been reported to correlate linearly with age (Friedheim, et al, Lancet, 1983). These authors suggest that choroid plexus acts as a protective sinkfor heavy metals. They also suggest that chelating agents may be useful in stroke survivors because of the reported correlation between lead exposure and CNS vascular disease.

To test these hypotheses, we used inductively coupled argon plasma emission spectroscopy to measure 19 trace elements and 4 major elements in choroid plexus of 18 random necropsies (ages 39-90). If the choroid plexus acted as a protective sink, then heavy metal concentrations should increase as a function of age. Chelation therapy should be recommended for stroke patients only if those with vascular disease have increased heavy metal concentrations in their choroid plexus.

We found that only patients over the age of 70 had lead concentrations in choroid plexus greater than 2 PPM. These concentrations correlated better with phosphorus content than with age. No age correlation was found for copper, iron or zinc. These data do not support the hypothesis that choroid plexus acts as a protective sink for heavy metals.

When patients with and without CNS vascular disease were compared, we found no differences between concentrations of lead, copper, iron or zinc in choroid plexus. Thus, we cannot support the use of chelating agents in the treatment of stroke survivors.

344.4 THE EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ON THE CONTRACTILE PROPERTIES OF REINNERVATED RAT MUSCLE. R.F. Mayer, E. Toyoshima and S.R. Max. Veterans Administration Medical Center & Dept. of Neurology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

We have studied the effects of 2,4-D on the isometric contractile properties of skeletal muscle during reinnervation after nerve crush.

Extensor digitorum longus muscles of adult male Fisher rats (160-260 g) were stimulated with indirect peroneal nerve and direct muscle electric shocks in vivo (38°C) at 1, 10, 17 and 24 days after crushing the peroneal nerve 1 cm from the muscle. The 2,4-D (100 mg/kg body weight, dissolved in emulphor/ethanol/H_2O) was injected i.p. 6 days/week for 24 days; controls received vehicle.

Functional reinnervation of the hind limb was observed 8-9 days after nerve crush, and complete recovery over 3 weeks was similar in 2,4-D and controls. Twitch tension (Pt) to direct stimulation changed little over the 24 day period in 2,4-D and controls, while Pt to indirect stimulation was 11% of intact values at 10 days, 58% at 17 days and 78% at 24 days in 2,4-D; recovery was similar in controls. Twitch/tetanus ratios (Pt/Po) to direct and indirect stimuli were increased (150%) at day 10 both in 2,4-D and controls, but at day 24, the Pt/Po remained 50% higher in 2,4-D than controls. Twitch tension expressed as grams force/g muscle weight (g/g) to direct (D) and indirect (I) stimuli was greater in 2,4-D (D, 7.1 g/g; I, 6.3 g/g) than in controls (D, 4.5 g/g; I, 3.8 g/g).

These changes indicate that 2,4-D does not interfere with the process of nerve regeneration and reinnervation of muscle following crush but it does produce a myopathy in which the twitch tension/g muscle weight is increased as is the Pt/Po suggesting both proliferation and disruption of myofilaments. (Supported by a grant from the Veterans Administration Research Service.)

344.5 EFFECT OF ACRYLAMIDE ON GAPDH ACTIVITY IN THE RAT OPTIC SYSTEM. M. I. SABRI and P. S. SPENCER, Institute of Neurotoxicology, Departments of Neuroscience, Neurology and Pathology, Albert Einstein College of Medicine, New York, N.Y. 10461.

The inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and neuron specific enolase by acrylamide, a potent neurotoxin, has been studied by several investigators. It has been suggested that acrylamide causes axonal degeneration by inhibiting the GAPDH-dependent axonal transport systems. Whether GAPDH inhibition, an event known to block axonal tranport in vitro, is the cause or the result of nerve-fiber degeneration in acrylamide neuropathy is unclear. We have examined the relationship between GAPDH inhibition and the onset of nerve-fiber degeneration in optic pathways and tibial nerves of acrylamide-treated animals. Male Sprague-Dawley rats (270-290 g) received acrylamide (35 mg/kg i.p.), 5 days/week for periods up to four weeks. Mild signs of acrylamide neuropathy (stiffened tails, foot splay, and hind-limb weakness) appeared after 14 days and, by 4 weeks, rats displayed severe hind-limb paralysis. Animals were decapitated after 1,2,3 and 4 weeks of acrylamide treatment; optic nerves and tracts were dissected, divided into 5 mm segments and stored at -80°C. In some experiments, tibial nerves were also sampled to compare the effect of acrylamide on GAPDH activity in the two systems. Tissue was homogenized in pyrophosphate buffer (pH 8.0), centrifuged at 10,000 x g for 2 min and GAPDH activity determined in the supernatant. Selected acrylamide-treated rats were systemically perfused with phosphate buffered glutaraldehyde and the nerves under study excised for neuropathological examination.

Preliminary results show that GAPDH activity in optic nerves and tracts is uninhibited for up to four weeks of acrylamide treatment. By contrast, there was a 35% reduction in GAPDH activity in sciatic nerves from the same animals. Light-microscope examination revealed nerve-fiber degeneration in tibial nerves and branches, but optic nerves and tracts from acrylamide-treated rats appeared normal. These results are consistent with our earlier data that acrylamide altered GAPDH activity in sciatic nerves but not in brain homogenates. The morphological and biochemical data obtained in this study confirm that peripheral nerves are more susceptible to acrylamide and that nerve-fiber change in optic tracts is a late event. Experiments are underway to study the proximal-distal gradient of GAPDH activity in the optic system and in sciatic nerves to examine more closely the relationship between GAPDH distribution and nerve-fiber degeneration in experimental acrylamide neuropathy. Supported in part by NIOSH 00851 and NINCDS NS 19611.

344.6 THE EFFECTS OF LEAD POISONING ON PYRAMIDAL CELL DENDRITE DEVELOPMENT OF MOTOR CORTEX IN THE NEONATAL RAT - A GOLGI STUDY. D. Lorton, W.J. Anderson and P. Kunkler*. Indiana Univ. Sch. Med., Terre Haute Ctr. for Med. Ed.; Indiana State Univ., Life Sciences Dept., Terre Haute, In 47809.

Rat pups from pregnant Long-Evans rats were given the standard dose 600 mg of lead acetate per kg of body weight (Press, 1977) every 24 hours beginning one day after birth until cumulative doses of 2400 mg/Kg (4 doses) were administered via stomach intubation. The brains were removed at 30 days of age and processed using a modified Golgi-Cox technique. The body weights of the lead treated rats were not significantly lower than control rats at 10 and 30 days of age. The brain weights of the lead treated group were significantly greater than the brain weights of control rat at 10 and 30 days. Camera lucida drawings of pyramidal cells from motor cortex of control rats contained significantly (54%) more secondary and tertiary branches extending laterally from the primary apical dendrite than pyramidal cells of leaded rats. No difference in the height of the pyramidal cells from lead treated and control rats was observed in the cortex. The dendritic branches were numbered according to their branching point away from the soma. There was a significant reduction in the number of 4, 5, 6 and 7 order branches extending from the apical dendrite and 3 and 4 order branches extending from the basal dendrites in the lead treated rats. The lengths of each dendritic branch at each order was measured for both apical and basal dendrites. The mean dendritic length was reduced by 19% in basal dendrites and by 28% in apical dendrites. Measurements of dendritic material by the Scholl method (1959) revealed similar results. Lead treated rats had a 17% reduction in the basal dendrites and an 36% reduction in the apical dendritic material. The number of spines per mu on pyramidal cells of experimental rats did not significantly differ from the number on pyramidal cells of the motor cortex of experimental rats at a point 15 mu above the soma. These results suggest that neonatal lead exposure alters the dendritic field in pyramidal cells of rat motor cortex and may reduce the ability of neurons to recieve and consequently integrate information. This may be responsible for the diminished learning capacities, hyperactivity, restlessness and the reduced adaptive behavioral patterns generally observed following lead poisoning.

344.7 Inhibition of Respiration by Inorganic Lead in Cultured Astrocytes and Neurons. D Holtzman and J Olson Dept of Neurol, Tulane Univ Sch of Med, New Orleans LA 70112

Inhibition of aerobic energy metabolism may be important in the pathogenesis of cellular Pb toxicity in the developing brain (Holtzman et al., Virch Arch 387:147, 1980). In situ and in culture, the neuron is more sensitive to Pb toxicity than the astrocyte (Nguyen et al., SEM, 1982:891). We now report the effects of Pb on respiration in neurons and astrocytes from primary culture.

Astrocytes and granular neurons were cultured from the neonatal rat brain (Olson and Holtzman, J Neurosci Res 5:497, 1980; Jameson et al. J Neurochem 42:470, 1984). For long-term exposures, lead acetate ($PbAc_2$) was added to the culture medium for 4 days beginning after 14 days (astrocytes) or 4 days (neurons) in culture. Respiration was measured polarographically in cell suspensions with glucose as substrate. For brief exposures, $PbAc_2$ was first added to cell suspensions in the respiration chamber.

After 4 days exposure to 1 μg/ml, the maximal respiratory capacity (dinitrophenol-stimulated respiration) was inhibited in neurons and astrocytes (27% inhibition in each cell type compared to controls, $p < .01$). Prolonged exposure to lower Pb concentrations did not affect cell respiration. When $PbAc_2$ was added (0.1-10 μg Pb/ml) to neuronal suspensions, the DNP-stimulated respiration also was inhibited by about 25%. Compared to neurons, $PbAc_2$ added to suspensions of astrocytes produced less inhibition of DNP-stimulated respiration at the higher concentrations (10% at 10 μg Pb/ml, 8% at 1 μg Pb/ml) and no inhibition at 0.1 μg Pb/ml. The high Pb (10 μg Pb/ml) produced no effects on respiration in suspensions of astrocytes pre-exposed to low Pb concentrations for 4 days in culture.

In summary, aerobic energy metabolism is inhibited by lower concentrations of Pb in neurons than in astrocytes from primary culture. This difference is consistent with the lower concentrations of Pb which alter surface morphology and viability in cultured neurons compared to astrocytes (Nguyen et al., 1982). It also supports the proposal that, both in vitro and in vivo, astrocyte resistance to Pb toxicity depends upon the capacity to sequester Pb in non-mitochondrial sites; i.e., lysosomes and nuclei (Holtzman et al., Neurotoxicology, in press). This capacity of the cultured astrocyte to sequester Pb may be increased by previous Pb exposure, perhaps by induced synthesis of a transport or binding protein. (Supported by NIH grant ES 03241).

344.8 EFFECTS OF TRIMETHYLTIN ON INCREMENTAL REPEATED ACQUISITION (LEARNING) IN THE RAT. M.G. Paule and D.E. McMillan*. Pharmacodynamics Br., Div. Terat. Res., Nat. Center Toxicol. Res., Jefferson, AR 72079 and Dept. Pharmacol., Univ. Ark. Med. Sci., Little Rock, AR 72205.

We administered the neurotoxicant, trimethyltin chloride (TMT) known to cause lesions in the hippocampus and assessed the effects of such treatment on learning in the rat. Adult male Sprague-Dawley rats were trained to perform an incremental repeated acquisition (IRA) task for food. At the beginning of each daily session (Monday through Friday), responses on only one of three levers produced food. After meeting criterion (40 errorless sequences) on one lever, the task was 'incremented' so that sequential responses on two levers were required and so on up to a maximum of five sequential responses. Each new required response was added in front of the previously performed sequence. Sequences of required lever presses changed daily. The effect of a single i.p. injection of known neurotoxic doses (3 rats received 7.5 and 2 received 8.7 mg/kg TMT) of trimethyltin chloride (TMT) on stable IRA behavior was studied. TMT administration resulted in alterations of both response efficiency and rate whereas vehicle (saline) injections had no effect on these parameters. Response efficiencies were decreased in all 5 subjects and these decrements were maximal 2-3 weeks after TMT injection for most animals. Performance recovered in 3 of these rats within 1-5 weeks; values for the other 2 animals did not return to pre-TMT levels for at least 3 months. Decreased efficiencies resulted from simultaneous increases in both within- and between-sequence errors. Increases in between-sequence errors took longer to recover than within-sequence errors. IRA response rates increased in one animal immediately after TMT injection and remained elevated for 5 weeks. Rate increases were noted in 3 of the other 4 rats but these changes were not seen until 2-4 weeks after TMT exposure. TMT effects on IRA behavior appear to follow the previously reported time course of lesion development in the rat hippocampus. These findings are consistent with the hypothesis that the hippocampus may have a role in learning phenomenon.

344.9 TRIMETHYLTIN (TMT) INDUCED ALTERATIONS OF MUSCARINIC RECEPTOR BINDING AFFINITY AND MONOAMINE TURNOVER IN MOUSE BRAIN. S.F. Ali, W. Slikker, Jr.* and G.D. Newport*. Pharmacodynamics Branch, Division of Teratogenesis Research, National Center for Toxicological Research, Jefferson, AR 72079.

Our laboratory previously reported that acute TMT administration produces a dose-dependent decrease in muscarinic receptor binding in mouse frontal cortex and hippocampus. In order to determine if the decrease was due to a change in receptor affinity or density, a Scatchard analysis was performed. Male C57Bl/6N mice, 8-10 weeks old were administered a single oral dose of 0, 1 or 3 mg/kg TMT-hydroxide dissolved in deionized water and sacrificed 2, 7 and 14 days later. The brains were quickly removed, dissected and frozen at -70°C for further analysis of muscarinic receptors over a (^{3}H)-Quinuclidinyl benzilate (QNB) concentration range of 0.02 to 2.0 nM. Two days after TMT treatment, the Kd for (^{3}H)-QNB in frontal cortex increased from 0.108 nM (control) to 0.291 nM (1 mg/kg) and 0.284 nM (3 mg/kg) while the number of receptors remained relatively constant (1407-control to 1496 - 1 mg/kg and 1591 - 3 mg/kg pmole/g of protein). A gradual return to control binding affinity was seen over the next two weeks. In the hippocampus a similar decrease in (^{3}H) QNB affinity was seen only at the 3 mg/kg dose 1 and 2 weeks after TMT treatment. In the caudate nucleus, we previously found a significant decrease in 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) concentrations 2 weeks after TMT treatment without any alterations in serotonin or dopamine concentrations. In order to determine if TMT altered metabolite efflux, mice were dosed with 0 or 3 mg/kg p.o. TMT-hydroxide. Two weeks later, pargyline (75 mg/kg i.p.) was administered and the mice were sacrificed at 0, 30 and 60 min. Concentrations of monoamines and their metabolites in the caudate nucleus were measured using HPLC/EC. Elimination rates of 5-HIAA and HVA were not altered by TMT treatment; therefore, the data suggest that the lower concentrations of monoamine metabolites previously seen 2 weeks after TMT treatment were due to a decrease in monoamine turnover. The decrease in muscarinic receptor affinity for (^{3}H)-QNB in frontal cortex and hippocampus and the decrease in the rate of serotonin and dopamine turnover in the caudate nucleus indicate the involvement of these three neurotransmitter systems in TMT-intoxication.

344.10 EFFECTS OF TRIMETHYLTIN IONTOPHORESIS ON MOUSE HIPPOCAMPAL SLICES. D.L. Armstrong, S. Farias*, M.J. Wayner, T. Williams*, and H.L. Read*. Division of Life Sciences, University of Texas at San Antonio, San Antonio, TX 78285

Trimethyltin (TMT) is a potent animal and human neurotoxin whose mechanism of action is unknown. Pathology studies have revealed necrosis in the pyriform cortex, amygdaloid nucleus, brain stem, and hippocampus following acute and chronic exposure. This selective toxicity suggests that TMT might be interacting with macromolecular components present only in certain neurons. The purpose of this study was to examine the responses of cells from different regions of the hippocampus to the direct iontophoretic application of TMT. Cells in regions most sensitive to the toxin would be expected to display consistent changes in electrical activity if TMT affects specific receptor or enzyme systems of these cells.

Four-barrel microelectrodes were used to apply 0.5 M trimethyltin chloride in 0.15 M NaCl, 1.0 M glutamate, and .15 M NaCl as a control ejection. The barrel used for extracellular recording contained 3 M NaCl. Granule cells in the dentate gyrus were unaffected by TMT or displayed excitation. The excitatory response was immediate and increased in magnitude and duration as ejection currents of 5, 10, 15 and 20 nA were sequentially applied. Pyramidal cells in the CA3 region of the pyramidal cell line displayed a decrease in spontaneous activity in response to TMT application using ejection currents of 5-20 nA. Initial samples of responses of CA2 and CA1 pyramidal cells indicates they are excited by TMT and more data is being collected on these regions.

If the inhibition of spontaneous activity of CA3 cells by TMT is mediated via a specific receptor interaction pharmacological agents should enable us to block or enhance these effects. Experiments are being carried out to characterize receptors on CA3 pyramidal cells that might mediate TMT's depressant effects.

344.11 FURTHER INVESTIGATIONS INTO THE INHIBITION OF OXIDATIVE ENZYMES BY ACRYLAMIDE (ACR). D. W. Sickles* and B. D. Goldstein. (SPON: M. J. Mulroy). Departments of Anatomy and Pharmacology and Toxicology, Medical College of Georgia Augusta GA 30912.

Acrylamide (ACR) monomer is a neurotoxic chemical which produces a central-peripheral distal axonopathy by an unknown mechanism. One hypothesis suggests inhibition of enzymes responsible for energy transformation as the key site of action. Acrylamide inhibits certain glycolytic enzymes as well as NADH-tetrazolium reductase (NADH-TR) following chronic dosing. The current study examines the time course of change in NADH-TR activity following a single 50 mg/kg dose of ACR. Eighteen adult (250-325g) male Sprague-Dawley rats were anesthetized with Brevital (60 mg/kg) 18-22 hours prior to sacrifice. The left soleus (SOL), composed of 84% slow oxidative (SO) muscle fibers, was injected with 3.5 µl of 25% horseradish peroxidase (HRP); the right tensor fascia lata (TFL), composed of 94% fast glycolytic (FG) fibers, was given 10.5 µl of the same HRP solution. A single 50 mg/kg intraperitoneal dose of ACR was given 0.5, 1, 6, 12, 24, 48, 72 or 96 hours prior to sacrifice. The spinal cords were removed and processed for histochemical demonstration of HRP and NADH-TR activity. Quantitation of the NADH-TR activity in each α-motoneuron was done with a Zeiss Zonax MPM03 microdensitometer, the results given as average optical density (O.D.) units (proportional to enzyme activity). The mean O.D. of soleus α-motoneurons decreased from control levels of 0.435 (n=41) to 0.345 (40) in 30 minutes and to a low of 0.273 (122) one hour after the single dose. The activity remained low, 0.283 to 0.304 (292), over 24 hours followed by a gradual return to normal levels of 0.42 (100) at 72 to 96 hours. The NADH-TR activity in FG motoneurons was not significantly changed. Therefore, ACR directly inhibits enzymes responsible for NADH oxidation; the selectivity for SO motoneurons is likely due to its normally high oxidative metabolism. These enzyme activity changes occur prior to any changes in axoplasmic transport suggesting a primary site of action. The reestablishment of normal enzyme levels indicates the synthetic machinery is not irreversibly damaged by ACR.

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344.12 ORAL POLYCHLORINATED BIPHENYL EXPOSURE INDUCES REGIONAL CHANGES IN BRAIN SEROTONIN METABOLISM. K.O. Brosch*, R.F. Seegal, and B. Bush* (Spon: J. Peck). Ctr. for Labs & Research, NYS Department of Health, Albany, NY 12201.

Polychlorinated biphenyls (PCBs) are widespread, persistent environmental contaminants that have been reported to cause neurological complaints in industrial workers and others exposed to PCBs (Fischbein, et al., Ann. NY Acad Sci., 320:703,1979; Chia, L. & Chu, E.L., Prog. Clin. Biol. Res., 137:117, 1984) and neurochemical changes in laboratory animals (Agrawal, A. et al., Toxicol. Lett., 7:417 1981). We report evidence of alterations in central serotonin metabolism in adult rats following a single oral exposure to a complex mixture of PCB congeners.

Adult male rats were orally gavaged with either corn oil (controls) or corn oil containing equal weights of Aroclors 1254 and 1260 calculated to yield a final dose of either 500 or 1000 mg/kg of total PCBs. Animals were sacrificed either 1,3,7 or 14 days after gavage, their brains rapidly removed and frontal cortex (FC), hippocampus (HC), hypothalamus (HT), lateral olfactory tract (LOT) and brainstem (BS) dissected free. Brain regions were homogenized in 0.2N perchloric acid containing 100mg/L EGTA and analyzed for serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) concentrations by high-performance liquid chromatography with electrochemical detection.

5-HT concentrations were significantly reduced in FC and HC, unaffected in HT and BS and elevated in LOT. 5HIAA/5-HT ratios were significantly elevated in all brain areas except for HT and LOT. Changes in 5-HT concentrations were most evident during the first several days following exposure to PCBs while 5HIAA/5-HT ratios remained elevated throughout the 14d experiment.

These results: (1) demonstrate that the mature nervous system is sensitive to a brief exposure to PCBs; and (2) further emphasize the need to examine several brain regions for neurochemical change in order to determine the action of putative neurotoxins.

344.13 ALTERATIONS IN NERVOUS SYSTEM-SPECIFIC PROTEINS, BEHAVIOR AND MORPHOLOGY ARE ASSOCIATED WITH THE DEVELOPMENT OF CEREBELLAR HYPOPLASIA IN THE GUNN RAT. J.P. O'Callaghan* and D.B. Miller. Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Toxicant-induced injury to the nervous system is associated with complex biochemical, functional and morphological responses. In order to characterize and quantify these responses, we are examining changes in nervous system-specific proteins (NSSP)(O'Callaghan and Miller, Trends Pharmacol. Sci. 4: 388, 1983), behavior and morphology that are associated with exposure to known neurotoxicants. In the present study, endogenous cerebellar neurotoxicity was assessed in the Gunn rat, an autosomal recessive mutant that exhibits degeneration of Purkinje cells and subsequent gliosis due to hereditary hyperbilirubinemia. NSSP measurements consisted of (1) G-sustrate, the endogenous substrate of cGMP dependent protein kinase that is specific to cerebellar Purkinje cells, (2) synapsin I, the endogenous substrate of cAMP dependent protein kinase that is associated with synaptic vesicles, apparently in all synapses and (3) glial fibrillary acidic protein (GFAP), a phosphoprotein specific to fibrous astrocytes. Quantification of these proteins and of cAMP and cGMP was achieved by radioimmunoassay or immunobinding to nitrocellulose (Jahn, et al. PNAS 81: 1684, 1984). Functional competence was assessed with measures of locomotor activity and a learning and memory task. Cerebellar weights and light microscopy were the indices of morphology. In comparison to values obtained from heterozygous (Jj) controls, the concentration of G-substrate and cGMP in the cerebellum of homozygotes (jj) was reduced by 77% and 35%, respectively, whereas cerebellar synapsin I and cAMP were unaffected. GFAP concentration in the cerebellum of jj rats was increased 380% above that of controls, a finding suggestive of an astrocytic response to excess bilirubin. Motoric competence of neonatal jj rats assessed in an open-field was decreased in comparison to corresponding Jj controls; as young adults jj rats were hyperactive when tested in a figure-8 maze. Performance of jj rats in an 8-arm radial maze did not reveal learning and memory deficits. Cerebellar tissue obtained from subjects with elevated serum bilirubin were reduced in wet weight and showed light microscopic evidence of Purkinje cell loss, findings consistent with the neurotoxic effects associated with hyperbilirubinemia in the Gunn rat. Our results demonstrate the use of a multiendpoint strategy for characterization and quantification of neurotoxicity.

344.14 EFFECTS OF METHYLPYRIDINES ON CENTRAL NERVOUS SYSTEM (CNS) EXCITABILITY AS INDEXED BY SEIZURE PARAMETERS. L.J. Burdette* and R.S. Dyer (SPON: M. Abou-Donia) Northrop Services Inc. and U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Although anticonvulsant effects of pyridine are documented, little is known about methylpyridine (MP) compounds, despite their prevalence as byproducts of coke operations and as organic intermediates in the manufacture of textile and pharmaceutical products. CNS excitability was assessed in MP-treated rats by studying pentylenetetrazol (PTZ) seizures and hippocampal afterdischarges (AD). Male Long-Evans rats were injected i.p. with saline or 100 mg/kg of 2-, 3- or 4-MP (n=20/group). Rats injected i.p. with 0, 100, 200 or 300 mg/kg pyridine (n=20/group) were included in PTZ studies. Testing was initiated one hour after dosing. Seizure latency, duration and severity were scored following a 50 mg/kg i.p. PTZ injection. Primary AD threshold, and the duration of the AD, the postictal depression and the rebound AD were determined in rats with bipolar electrodes implanted in the dorsal hippocampus. The anticonvulsant properties of pyridine were confirmed, as indicated by the absence of seizures and the significantly increased seizure latency and decreased seizure duration and severity exhibited by rats dosed with 300 and 200 mg/kg, respectively. Similarly, acute exposure to 2- and 3-MP increased seizure latency and decreased seizure severity; acute 4-MP treatment did not affect PTZ seizures. Although AD parameters were unchanged by any of the MP compounds, the presence of unusually complex spike activity during the afterdischarge (rated by two judges blind to experimental condition) was significantly more likely following acute 3- or 4-MP exposure. The data indicate that 2- and 3-MP not only share some CNS depressant properties with pyridine, but also may be more potent since effects were observed at a lower dosage.

344.15 PYRIDINE AND METHYLPYRIDINES AFFECT BRAINSTEM AUDITORY EVOKED RESPONSES. Raelyn Janssen* and Robert S. Dyer (SPON: Michael I. Gage). Neurophysiology Branch, Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Pyridine (PYR) has central nervous system (CNS) depressant properties, but its effects on sensory evoked potentials have not been reported. Less is known about the effects of methylpyridine compounds on the nervous system, despite a yearly production volume of about 1 million lbs. We investigated the effects of acute administration of PYR and 2-, 3-, and 4-methylpyridine (2MP, 3MP, 4MP) on brainstem auditory evoked responses (BAERs) in rats.

Long-Evans rats were implanted with skull screw electrodes and tested without anesthetic a week after surgery. The test compound, or saline, was administered i.p. 1 hour before testing. Dosages were: (PYR) 0, 100, 200 or 300 mg/kg; (2MP, 3MP, 4MP) 0 or 100 mg/kg. Click stimuli of 50 μs duration were presented at a rate of 10/s and at a level of 90 dB max rms spl. Latencies and amplitudes of the waveforms resulting from averaging 512 responses were recorded. Multivariate Bonferroni-corrected analyses were performed on: (1) peak latencies, (2) interpeak latencies (brainstem conduction time), and (3) peak-to-peak amplitudes.

The 300 mg/kg dose of PYR significantly prolonged latencies of all centrally-generated (brainstem) peaks without affecting latencies of peaks representing peripheral function (inner ear or auditory nerve). This central effect was also reflected in interpeak latencies. Amplitudes of a peripheral peak and several central peaks were increased. All three MP compounds increased amplitudes of some of the central peaks, although the peaks affected varied across compounds. 2MP prolonged peripheral and central peaks and interpeak latencies. There was a non-significant trend toward prolonged latencies in the 3MP group, and interpeak latencies were significantly increased. 4MP did not affect either peak or interpeak latencies.

2MP and 3MP, like PYR, produced latency increases, which are signs of CNS depression. The presence of methyl groups prevented the peripheral amplitude increase seen with PYR. On the other hand, PYR left peripheral latencies unchanged, whereas 2MP prolonged them, and 3MP produced a trend toward prolonged latencies. Thus, the nature of depression varied with the compound.

NEUROTOXICITY IV

345.1 INHIBITION BY ACRYLAMIDE OF PERIKARYAL RESPONSES TO SCIATIC NERVE TRANSECTION. M. S. Miller and P. S. Spencer, Institute of Neurotoxicology, Depts. of Neuroscience and Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

Repeated exposure of humans or laboratory animals to monomeric acrylamide (ACMD) results in a central-peripheral distal axonopathy which involves long, large-diameter sensory and motor axons. There is strong evidence that alterations in axonal transport may underlie ACMD-induced axonopathy. Repeated administration of ACMD results in marked deficits in both anterograde and retrograde axonal transport. Single doses of ACMD, equivalent to repeated doses that precipitate neuropathy, produce dose-dependent deficits in the rate of retrograde transport in sensory and motor axons. These changes in retrograde axonal transport precede the onset of functional or morphologic signs of neuropathy. Several studies have indicated that retrograde transport may be a means by which perikaryal responses to peripheral axon lesions are initiated. Thus, ACMD may impair regenerative functions of neuronal perikarya, possibly by disrupting a retrogradely transported signal which normally activates the reparative response. The ultimate effect of altered retrograde axonal transport would then be to impair axon repair and maintenance processes, leading to axonal compromise and degeneration.

This hypothesis is addressed by the measurement of ornithine decarboxylase (ODC, EC 4.1.1.17) activity and rate of total RNA synthesis in L_5 dorsal root ganglia (DRG) following induction of non-specific axon damage by unilateral sciatic nerve transection. Within 24 h of nerve transection, ODC activity in ipsilateral DRG increased approximately 25-fold from values for contralateral DRG. Induction of ODC activity was maximal 48 hours post-axotomy (approx. 40-45 fold induction), and was still elevated at 9 days post-axotomy. ODC activity in contralateral DRG was unchanged at all times measured. Rate of ^{3}H-uridine incorporation into the trichloroacetic acid-insoluble fraction of axotomized neuronal perikarya was increased 2.4-fold. Single and repeated dosing with ACMD monomer (50 mg/kg, ip) resulted in marked attenuation of the induction of perikaryal ODC activity and rate of ^{3}H-uridine incorporation associated with nerve transection. Additional data suggest that ACMD does not act directly on perikarya of afferent neurons in DRG to inhibit induction of ODC following axotomy.

These data are consistent with the hypothesis that ACMD-induced central-peripheral distal axonopathy is the result of impaired or misdirected repair processes, rendering neurons incapable of initiating appropriate responses to sub-lethal axon damage.

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345.2 INFLUENCE OF CHRONIC EXPOSURE TO INORGANIC LEAD ON VALPROATE-INDUCED GAMMA-AMINOBUTYRIC ACID ACCUMULATION. S. M. Lasley. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

Previous studies (Silbergeld, E. K. et al., J. Neurochem. 34:1712, 1980; Memo, M. et al., Toxicol. Lett., 6:427, 1980) have suggested a role for gamma-aminobutyric acid (GABA) in the CNS neurotoxicity resulting from chronic developmental exposure to inorganic lead (Pb), but a direct assessment of turnover has not been made. This study examined the regional GABA accumulation in exposed and control rats resulting from the administration of valproic acid (VPA), an inhibitor of GABA metabolism whose action is relatively specific for nerve endings (Gale, K. and Iadarola, M. J., Science 208:288, 1980).

At parturition Long-Evans dams received 0.2% Pb acetate in the drinking water thus exposing the suckling pups to Pb via maternal milk. Control dams received distilled water. Pups were weaned to, and maintained on, the same solution given their dam until sacrifice. At 150-170 days rats were injected with either 400 mg/kg i.p. VPA or isotonic saline, sacrificed by the near-freezing method, and brains removed and dissected in a cryostat at -20°C. Tissue samples were derivatized by o-phthaldialdehyde and beta-mercaptoethanol and analyzed by liquid chromatography with gradient elution and fluorescence detection to determine amino acid content.

Blood Pb values determined at 90 days of age were 55.6 ± 12.5 and 3.8 ± 2.1 μg/dl in exposed and control animals, respectively (mean ± SD, N = 16 for each group). No Pb-induced changes were observed in three brain areas rich in GABA synapses - substantia nigra, globus pallidus, ventral tegmental area - as a result of either exposure alone (saline animals) or in conjunction with the administration of VPA. Neither were exposure-related changes found in aspartate, glutamate or taurine concentrations in these regions. Since the GABA neurons examined are major components of brain pathways subserving motor activity, these findings suggest that chronic exposure to low amounts of Pb via the lactating dam model does not result in alterations in neuronal activity in these pathways, in agreement with recent investigations reporting no effect of chronic exposure on spontaneous locomotor activity employing the same exposure protocol (Zenick, H. et al., Toxicol. Appl. Pharmacol., 64: 52, 1982). (Supported in part by NIH grant ES01566).

345.3 2,4-DITHIOBIURET DEPRESSES TRANSMITTER RELEASE AT THE RAT NEUROMUSCULAR JUNCTION. M.H. Weiler and R.E. Peterson*. University of Wisconsin-Madison, School of Pharmacy, Madison, WI 53706.

2,4-dithiobiuret (DTB) induces an ascending muscular weakness in rats. It has been demonstrated through studies of skeletal muscle contractile responses to direct and indirect stimulation that the effects of DTB are prejunctional and apparently cause a reduction in acetylcholine (ACh) release at the neuromuscular junction (Atchison et al., Neurotox., 2: 329, 1981). The purpose of the present investigation was to evaluate the effects of DTB on synaptic transmission in the extensor digitorum longus (EDL) of the rat. Rats were treated daily with DTB (1 mg/kg/day X 6 days, i.p.) or 0.9% NaCl (1 ml/kg/day X 6 days, i.p.). On the sixth day, the EDL and 1 cm of associated peroneal nerve was dissected and placed in an oxygenated physiological saline (pH 7.3, 37°C). Miniature endplate potentials (m.e.p.p.s) and evoked endplate potentials (e.p.p.s) were recorded intracellularly with 3 M KCl-filled microelectrodes. The frequency ($\bar{X} \pm S_E$.) of m.e.p.p.s in DTB-treated EDLs was 0.50 ± 0.06 sec^{-1} (n=13), a rate which was significantly less ($p < .001$) than that of the control muscles (0.98 ± 0.11 sec^{-1}; n=14). There were no DTB-related changes in muscle membrane resistance or in resting potential. Statistical analysis of e.p.p.s measured in curarized (10^{-7} M) preparations indicated that the mean quantal content (m) was significantly depressed in the DTB-treated muscles. At stimulation rates of 1, 10, 20 and 50 Hz the estimated values of m in the DTB-treated preparations were, respectively, 21% (n=4), 25% (n=8), 45% (n=8) and 51% (n=5) that of the estimated quantal content of the controls. It is concluded that spontaneous and evoked transmitter release are depressed in DTB-treated rats. (Supported by NIH Grant ES01906 and AG01572.)

345.4 INVOLVEMENT OF DOPAMINERGIC AND SEROTONERGIC SYSTEMS IN TRIETHYLLEAD NEUROTOXICITY. D.L. DeHaven, D.W. Schulz, T.J. Walsh* and R.B. Mailman. Biol. Sci. Res. Ctr. and Curr. in Neurobiol. and Toxicol., Univ. North Carolina School of Medicine, Chapel Hill, NC 27514.

Triethyllead (TEL) is an organometal that produces a variety of neurobehavioral alterations. The present series of experiments attempted to elucidate the neurochemical sequelae following exposure to TEL.

Male Fischer-344 rats were administered a single dose of TEL (7.88 mg/kg, sc) or vehicle (distilled water) and were sacrificed by decapitation at 7 days post-dosing. Striatum, nucleus accumbens, olfactory tubercle, frontal cortex and hippocampus were rapidly dissected and regional concentrations of NE, DA, DOPAC, HVA, 5-HT and 5-HIAA were assessed. In addition, binding to ^{3}H-spiperone receptors in striatum, ^{3}H-5-HT receptors in hippocampus, and DA-stimulated adenylate cyclase activity in striatum, frontal cortex and olfactory tubercle were determined.

Treatment with TEL significantly decreased the concentrations of DA in hippocampus and DOPAC in striatum. Concentrations of NE were decreased in hippocampus and frontal cortex. Serotonin levels were decreased in striatum and frontal cortex, and 5-HIAA concentrations were decreased in hippocampus and elevated in nucleus accumbens. The B_{max} for DA receptors in striatum was elevated 26% in TEL-treated rats, while serotonin receptors were unchanged. Finally, the V_{max} for DA-stimulated adenylate cyclase activity was elevated in olfactory tubercle and decreased in striatum, while the K_m was slightly increased in olfactory tubercle.

These data indicate that TEL primarily affects dopaminergic systems in the brain, and that these effects may be related to the behavioral alterations that occur following exposure to TEL. (Supported in part by ES-01104 and ES-07126)

345.5 PHARMACOLOGICAL PROBES OF HIPPOCAMPAL DAMAGE PRODUCED BY NEONATAL TRIETHYL LEAD EXPOSURE. R.M. Booze, H.A. Tilson*, S.C. Bondy, Z. Annau, and C.F. Mactutus. Lab. Behav. Neurol. Toxicol., NIH-NIEHS, Research Triangle Park, NC 27709, and The Johns Hopkins University, Baltimore, MD 21205.

Hippocampal lesions produce alterations in a number of brain regions, particularly in the dopaminergic basal ganglia systems and the cholinergic septal-hippocampal pathway. As neonatal triethyl lead (TEL) exposure produces relatively selective hippocampal damage, we examined the functional status of dopaminergic and cholinergic systems.

Offspring of Fischer-344 dams were neonatally exposed to TEL chloride. At five days of age, one pup of each sex per litter was administered a s.c. injection of either distilled water, 4.5- or 9.0-mg/kg TEL. The fourth pup of each sex was an undernourished control animal. Upon maturation to adulthood, animals were habituated to activity chambers over four days. Baseline activity differences were evident as the female TEL-treated animals were more active in a dose-dependent manner than control animals. All animals were then administered 0.1 and 1.0-mg/kg apomorphine in a cross-over design. Stereotypic behavior produced by apomorphine was not affected by the early TEL-treatment. In addition, although apomorphine significantly altered locomotor activity, there were no TEL-induced alterations in drug sensitivity. Two weeks after apomorphine testing, 1.0-mg/kg scopolamine and 1.0-mg/kg methscopolamine were given in a cross-over design. Activity testing revealed a differential effect of the two drugs as scopolamine increased activity, whereas methscopolamine decreased activity. Only the TEL-exposed female animals were increased in sensitivity to scopolamine independent from early undernutrition effects, peripherally-mediated effects, or baseline differences. In contrast to the activity data, both scopolamine and methscopolamine had similar effects on auditory startle responsiveness. Following scopolamine testing, the animals were sacrificed for receptor binding assays. Increased hippocampal ^{3}[H]-QNB binding was associated with the increased behavioral sensitivity of TEL-treated animals.

In summary, neonatal TEL-exposure was found to produce long-term alterations in behavioral reactivity. However, these alterations do not appear to be modulated by dopaminergic systems, but may be modulated by cholinergic manipulations in a gender-specific and task-specific manner. Receptor binding assays further confirmed the alterations in cholinergic system functioning.
(Supported in part by ES 07094)

345.6 EEG CORRELATES OF TRIMETHYLTIN NEUROTOXICITY. J.Kinsora*, J.French, J.G.Marriott and D.Robertson* (SPON:P.Poschel), Pharmacology, Warner-Lambert/Parke-Davis, Ann Arbor, MI 48105.

Selective damage to hippocampal pyramidal cells has been reported following acute exposure to trimethyltin (TMT). These studies have shown that morphological changes are maximal 10 days after dosing. The present study examined the EEG correlates of TMT induced neuronal damage.

Male, Long-Evans rats were chronically implanted in cortex, hippocampus and striatum with bipolar recording electrodes. Following 2 weeks of recovery, baseline EEG recordings were obtained. A computer was used to determine and evaluate the power spectrum associated with the EEG changes produced by TMT. An oral dose of 3 mg/kg TMT was then given to 6 rats over 3 consecutive days. Brain electrical activity was evaluated over 17 days post TMT, recording every other day for 1 hour. Each sample consisted of 1024 points of digitized EEG data obtained over 12 secs and 3 such samples were Fourier transformed and then averaged to give an estimate of 36 secs of the EEG spectra from each channel, approximately every 15 min.

From day 1 to day 7 post-dosing, increasing activity in the theta band (4-6 Hz) and decreasing activities in the delta (0-4 Hz) and beta (16-50 Hz) bands were observed in the hippocampus. No similarly progressive changes were found in the cortex or striatum. Theta activity, which dominated the post TMT hippocampal records, was blocked by an injection of atropine (50 mg/kg IP) on day 3 and day 7 for about an hour. Only 3 animals survived beyond day 7 and recordings from these animals continued for 10 more days. The amplitude of the hippocampal EEG declined steadily from day 7 until about day 11. On day 17, the surviving animals were perfused intra-cardially with saline followed by a 10 % formaldehyde solution. Subsequent histology revealed all electrodes were at the intended locations. Light microscope evaluation (250X) found complete destruction of the CA1 pyramidal cell area but no damage was evident in cortex or striatum.

The results indicate that graded changes in hippocampal EEG are correlated with TMT induced neurotoxicity. The increase in hippocampal theta activity may be related to an excitotoxic mechanism and its sensitivity to atropine suggests cholinergic involvement in the neuronal damage produced by TMT.

345.7 EFFECTS OF METHYLMERCURY ON NUCLEOSOMES OF HUMAN AND MOUSE FETAL ASTROCYTES IN VITRO: A STUDY WITH FLUORESCENT PROBE. B. H. Choi, L. A. Tengelsen* and H. Simpkins*. Dept. of Pathology, Univ. of California Irvine, Irvine, CA 92717.

It is widely recognized that methylmercury (MeHg) is highly neurotoxic and has a greater affinity for the developing central nervous system than that of adults. Neurological damage occurs in human fetuses when exposed to MeHg in utero. The pathogenesis of the neurological damages is largely unknown. The purpose of this investigation was to study the interaction of MeHg with nucleosomes of human and mouse fetal astrocytes in culture with the fluorescent probe, N-(3-pyrene)maleimide, which is specific for histone H3.

Pure monolayer cultures of human fetal astrocytes (HFA) were established from cerebral cortex of an 11-week-old fetus and maintained for more than 2½ years in culture. Mouse fetal astrocytes (MFA) were similarly propagated from fetal mice at day 16 of gestation. The cultures were exposed to 0.01 mM methylmercuric chloride (MMC) for 6 hours along with controls. After the incubation the cells were harvested by trypsinization and nucleosomes were prepared. The nucleosomes were then reacted with 0 to 200 nM N-pyrene maleimide for 25 minutes at room temperature. The proteins of the treated and control nucleosomes were analyzed by SDS polyacrylamide gel electrophoresis.

A consistent decrease (25 - 35%) in the N-(3-pyrene) maleimide fluorescence of the MMC-treated nucleosomes was observed both in human and MFA when compared with the controls. Exposures to 0.1% SDS for 15 minutes caused an increase of fluorescence in the control groups, however, the MMC-treated nucleosomes showed no change in the fluorescence pattern. These data indicate that MeHg is co-valently bound to histone H3 of nucleosome in highly specific manner when exposed to fetal astrocytes.

(supported in part by NIEHS Grant ES 02928)

345.8 CORTICAL DEMYELINATION IN NEWBORN KITTENS AFTER LOW-DOSE X-IRRADIATION. W.J. Anderson. Indiana Univ. Sch. Med., Terre Haute Ctr. for Med. Educ., 135 Holmstedt Hall, Terre Haute, IN 47809.

In a previous report (Anderson & Stromberg, 1977), our laboratory reported very briefly of a severe demyelinization in kittens irradiated at birth and at one week of age. This report was based upon kittens who died of seizures prior to age 70 days, the age at which all other kittens were sacrificed. At that time we hypothesized that those changes were due to disruption of gliogenesis at those early ages. This report is based upon a continuation of our earlier findings utilizing kittens 70 days old. This study utilized low level x-irradiation at weekly intervals beginning at birth. Six fractionated doses of 200R and 150R were given over a period of two weeks for each age group. Radiation treatment groups consisted of both whole-head and localized cortical radiation. Our results indicate that all animals irradiated at birth showed severe demyelinization of the cortical white matter with the posterior occipital cortex having leukomalacia cavitation in the white matter. An analysis of ten kittens, 70 days of age, displayed identical patterns of degeneration with only the frontal cortex showing any variation. Microscopic analysis revealed no alterations in any arteries or capillaries, but distended veins were found in areas of leukomalacia. Patterns of normal myelinated fibers adjacent to demyelinated fibers were found in many gyri, and especially in the corpus callosum. Normal numbers of glia were found in the myelinated fibers, while a sparcity of glia were found in demyelinated areas. At earlier ages a similar replication of this demyelination was present. An analysis of a separate series of kittens who received local cerebellar irradiation revealed no cortical demyelinization indicating that the x-ray and not the anaesthetic was the toxic agent. The one-week experimental group revealed minor demyelinization in four out of ten kittens. No demyelinization was seen in any other age group. It is our conclusion that the demyelinization was due to damage of presumptive stem oligodendroglia cells which result in a permanent condition of demyelinization.

345.9 NICOTINE BEHAVIORAL EFFECTS IN RATS WITH KAINIC ACID LESIONS OF THE NUCLEUS BASALIS. D.M. Benson and C. Ksir. Dept. of Psychology, Univ. of Wyoming, Laramie, WY 82071.

Rats with kainic acid lesions of the nucleus basalis have previously been reported to demonstrate an enhanced locomotor stimulation effect of nicotine compared to control rats (C. Ksir & D.M. Benson, Psychopharmacology, 81, 272-273, 1983). Measurements of nicotine effects on activity were based on data from photocell cages. The current experiment employed detailed observations of each rat in an effort to further characterize the different behavioral effects of nicotine in kainic acid lesioned rats.

Six adult male hooded rats were anesthetized and given injections of kainic acid (0.15 ug in 0.5 ul in each side) in the region of the nucleus basalis of Meynert (1.0 mm caudal to bregma, 3.0 mm lateral to midline, and 7.6 mm below the skull surface). Six other rats were given similar injections of the artificial CSF vehicle. Beginning 9 days after surgery, each rat was placed in a 50 cm cube made of clear acrylic with a white paper floor and was observed for 20 minutes. Observation sessions were repeated twice per week for each rat. Immediately prior to each session the rat was given an injection of either saline or of nicotine sulfate (0.1, 0.2 or 0.4 mg/kg, s.c.). Half the rats in each group were given the injections in order of ascending concentration, the other half in descending concentration. The observer did not know which dose was injected for each test. Behaviors were recorded in real time on a microcomputer keyboard.

Nicotine produced dose-related decreases in rearing and sniffing at restricted areas of the floor or walls in both groups. Dose-related increases in air sniffing were seen in both groups. Nicotine increased freezing behavior in the control group and exploration in the kainic acid lesion group. At the highest dose of nicotine the two groups showed significantly different amounts of these two behaviors. Thus, after nicotine the kainic acid rats spent a larger amount of time engaging in forward locomotion with the body extended, and usually accompanied by sniffing.

Rats with similar lesions were tested in photocell activity cages, in which 0.2 mg/kg nicotine produced a greater activation in the kainic acid group. This behavioral activation was blocked by 1.0 mg/kg mecamylamine given 15 minutes prior to the nicotine. These results indicate that rats with kainic acid lesions of the nucleus basalis are differentially sensitive to the behavioral effects of nicotine acting at classical nicotinic receptors.

345.10 DRAMATIC NEUROTOXICITY OF NEONATAL INTRACEREBRAL INJECTION OF TUBULIN-BINDING AGENTS. R. H. Dyck, I. Q. Whishaw, & R. J. Sutherland, Dept. of Psychology, Univ. of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

Intracerebral microinjection of microtubule disrupting agents can cause blockade of axonal transport processes and cell death in certain susceptible neuronal populations. Several studies have noted that granule cells in the hippocampal formation, cerebellum, and olfactory bulb degenerate following exposure to colchicine. Different neuronal populations within these and other structures survive similar dosages. In the course of studies of behavioural effects of hippocampal granule cell destruction, we have found that intracerebral microinjections of colchicine have a dramatically greater, and less preferential, neurotoxicity during the immediate neonatal period.

We conducted four experiments to examine the susceptibility of neonatal rat brain to the neurotoxic effects of tubulin binding agents. We tested the effects of: 1. varying the dosage of colchicine injected into the hippocampal formation in adults and neonatal rats, 2. varying the injection site within the neonatal forebrain, 3 varying the age at which colchicine is injected, and 4. the relative potencies of colchicine, B-lumicolchicine, vincristine, and vinblastine were assessed in neonates.

In neonates, we found that as little as 1.0 µg of colchicine causes degeneration of the entire forebrain and that 15 ng causes clear neuronal degeneration in the hippocampus and neocortex. There did not appear to be any differential susceptibility at the sites tested. The susceptibility to colchicine-induced cell death declines with age. Finally, we found that in terms of relative neonatal neurotoxicity the ranking was vincristine > colchicine > vinblastine > B-lumicolchicine.

This phenomenon has important implications for processes in postnatal neuronal development and has potential for use as a technique for investigating behavioural consequences and neuronal reorganization following neonatal brain damage.

345.11 **CONDUCTION PROPERTIES AND POTASSIUM CHANNEL BLOCKADE IN DEMYELINATED PERIPHERAL NERVE.** E.F. Targ* and J.D. Kocsis, Dept. of Neurology, Stanford University Medical School and Veterans Administration Medical Center, Palo Alto, CA 94304. In addition to impulse blockade and slowing, a number of hyperexcitability events have been suggested for demyelinated axons. Intra-axonal and whole nerve recordings were used to evaluate these events in isolated demyelinated nerves. We induced demyelination by lysolecithin (LPC) microinjection into rat sciatic nerve, and removed a 1-2 cm segment 5-7 days later. The ends of the nerve were placed across bipolar electrodes used for either stimulation or recording. The focal LPC lesion was positioned in the center of a recording chamber superfused with Ringer solution. The monophasic compound action potential recorded from normal nerve in our chamber consists of a discrete large amplitude negativity. Recordings through a site of demyelination are reduced in amplitude, with multiple components lasting over 10ms. The refractory period was increased in the demyelinated nerves, and the frequency-following ability of the late component was much attenuated as compared to normal areas of nerve. Single axon recordings were obtained using glass microelectrodes. For a given impalement, latencies were short (0.1-0.3ms) for propagation through the nondemyelinated segment, while much longer (4-12ms) through the lesion. The absolute refractory period for single axons, recorded through the lesion, was twice that for normal nerve segments. Spontaneously active axons, or multiple discharge following a single impulse were not seen in demyelinated nerve, nor did we find evidence for cross-talk or impulse reflection, which had been predicted for regions of demyelination. With application of the potassium channel blocker 4-aminopyridine (4-AP) to the lesion site, there was an enhancement in the trans-lesion compound response. Application to non-demyelinated segments of the same nerve had little effect. Although 4-AP increased the amplitude and duration of the trans-lesion response, the frequency-following capabilities of the slow response component were reduced, with individual axons remaining refractory for more than 20ms. Following 4-AP application, spontaneous firing and multiple spike discharge were present. Our results indicate that the primary conduction abnormality in isolated demyelinated peripheral nerve is conduction block and slowing. Hyperexcitability as evidenced by spontaneous activity and multiple discharge were not seen unless 4-AP was applied, however, in the presence of 4-AP, the frequency-following ability was reduced. These results stress the importance of potassium conductance in modulating impulse activity in demyelination.
Supported in part by a grant from the NIH, and the Medical Research Service of the V.A.

345.12 5-AMINO-2,4-DIHYDROXY-α-METHYLPHENETHYLAMINE PROVIDES MORE SELECTIVE NORADRENERGIC DESTRUCTION THAN 6-HYDROXYDOPAMINE. P. Lin*, R. Rhagavan*, R.E. Lehr*, R.M. Kostrzewa, S. Sasa*, and C.L. Blank. Dept. of Chemistry, University of Oklahoma, Norman, OK 73019.

We have recently completed the synthesis of the eight different 6-hydroxydopamine derivatives in which the ring is substituted at the 2,4, and 5 positions with trihydroxy or aminodihydroxy substituents and the side chain is either ethylamine or α-methylethylamine. Preliminary testing by both biochemical and histofuorescence has shown a few of the derivatives to be more selective than 6-hydroxydopamine when used for noradrenergic destruction.

When using a dose of 10 µg(free base, intracerebral, mouse) of either 5-amino-2,4-dihydroxy-α-methylphenethylamine(I) or 6-hydroxydopamine(6-HDA) the following results were obtained:

	Neurotransmitter, % Controls (± SEM)		
Neurotoxin	NE	DA	5-HT
I	26±3***	101±4	103±5
6-HDA	40±8***	86±1***	93±8
Controls	100±8	100±2	100±4

*** $P < 0.001$ compared to controls.

Thus, it appears that this compound(I) is superior to 6-HDA in its ability to effect noradrenergic destruction. It provides equivalent or greater loss of NE neurons than 6-HDA while not effecting the DA or 5-HT neurons.

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346.1 COMPUTER CONTROLLED SIMULATION OF D-AMPHETAMINE SELF-ADMINISTRATION PATTERNS. W.H. Lyness, A.P. Leccese and J.H. Pirch. Department of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX, 79430.

A computerized drug-delivery system capable of simulating the self-administration pattern displayed by animals given access to any self-administered drug is described. Rats allowed to self-administer d-amphetamine by activation of a pneumatic device (Weeks, PB&B, 7, 559, 1977) which delivers 0.125 mg/kg/inj d-amphetamine will press an operant lever an average of 15 times during the first half hour and then 6 to 8 times per hour for the remaining 7½ hours of the 8 hour daily session. A Timex Sinclair 1000® microcomputer was linked via a Byte-Back® control module and a standard 44 pin edge-connector to a set of twelve pneumatic syringes and was programmed to simulate the pattern of d-amphetamine self-administration displayed by rats. Thus, the device administers 15 injections at 2 minute intervals and then administers an injection every 8 or 10 minutes. The program for this particular pattern of administration is provided as well as a modification which enables the computerized apparatus to be used for self-administration studies. Electrical diagrams are provided to elucidate the computer-apparatus interface for both computerized delivery and self-administration application. The device enables study of the effects of chronic drug administration without assuming that the effects of a single large injection simulate those of chronic small doses. Data are presented which demonstrate marked differences in the recovery of brain monoamines and metabolite levels subsequent to computerized drug delivery and a single large bolus of d-amphetamine.

346.2 ALPHA-METHYL-PARA-TYROSINE BLOCKS METHAMPHETAMINE-INDUCED DEGENERATION IN THE RAT SOMATOSENSORY CORTEX. D.L. Commins* L.S. Seiden and C.R. Schuster (SPON: P.C. Hoffmann). Dept. of Pharmacol. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

A single high dose of methamphetamine (MA) (100 mg/kg,sc) produces signs of neuronal degeneration in the rat somatosensory cortex. When stained by the Fink-Heimer method, the affected area contains degenerative debris of either dendritic or axonal origin surrounding scattered argyrophilic neurons. The affected perikarya are most often located in lamina III of somatosensory cortical area 2 as defined by Krieg (J. Comp. Neurol. 84:241, 1946). Most of the degenerative debris is located dorsal to the affected perikarya in laminae II and III. However, thin degenerating processes (probably axons) are often found in laminae IV and V, running parallel to the cortical laminae. Pretreatment with alpha-methyl-para-tyrosine (AMT), a catecholamine synthesis inhibitor, blocks the long-term neurochemical (Wagner et al., Brain Res. 179:285, 1983; Hotchkiss and Gibb JPET 214:157, 1980) and histological changes (Ricaurte et al., Brain Res., in press) produced in the caudate by administration of MA. These neurochemical deficits include long-term dopamine depletions and suppression of both tryptophan hydroxylase and tyrosine hydroxylase activities. In the present study, the ability of AMT to prevent MA-induced neuronal degeneration in the somatosensory cortex was assessed. Rats received injections of AMT (150 mg/kg, sc) or saline one hr prior and six hrs subsequent to a single injection of either MA (100 mg/kg, sc) or saline. The subjects were perfused two days later. Brain sections were stained by the Fink-Heimer method followed by counter-staining with cresyl violet. Neurodegenerative changes were present in the somatosensory cortex of all animals that received MA alone. Treatment with AMT markedly attenuated the effect of MA in both cell bodies and processes. These findings suggest that the MA-induced degeneration observed in the somatosensory cortex is mediated via a catecholaminergic or serotonergic mechanism. Catecholaminergic and serotonergic neurons have not been reported to exist in the cerebral cortex. Thus MA may produce neuronal degeneration in the cortex by acting on catecholaminergic or serotonergic neurons presynaptic to the affected cortical cells.
This research supported by PHS DA-00250; D.C.: 5T32-MH-14274 Training; L.S.: RSA MH-10562.

346.3 6-HYDROXYDOPAMINE IS FORMED FROM DOPAMINE IN VIVO AFTER ADMINISTRATION OF METHYLAMPHETAMINE. L.S. Seiden and G. Vosmer. Univ. of Chicago, Dept. Pharmacol. and Physiol. Sciences, Chicago, IL 60637.

Administration of methamphetamine (MA) in large doses or for prolonged periods of time causes irreversible depletion of dopamine (DA) in several rat brain areas due to the degeneration and loss of DA nerve terminals (Ricaurte et al., Brain Res., in press). The mechanism of MA-induced DA neuronal toxicity is not understood. The neurotoxic effects of MA may result from the non-enzymatic conversion of DA to 6-OHDA since DA can be non-enzymatically converted to trihydroxyphenethylamines, among which 6-OHDA is a possible oxidative metabolite (Senoh et al., J. Amer. Chem. Soc. 81: 6236-40, 1959). In the first experiment, male rats were injected with MA (100 mg/kg s.c.) or saline and sacrificed at 0.5, 1, 1.5, 2, 4, 8, 16, and 24 hrs after injection. Amine analysis was done with high performance liquid chromatography with electrochemical detection. Rats treated with MA formed 6-OHDA in the caudate nucleus between 0.5 and 2 hrs after injection. The values for 6-OHDA were 0.5 hrs: 0.20 ± 17; 1 hr: 39 ± .31 and 2 hr: 24 ± .21, ng/mg tissue. In a second experiment, rats were injected with 6-OHDA (150 ug, ivt.) and sacrificed at 0.5, 1, 2, and 4 hrs after injection. Here the levels of 6-OHDA were of the same magnitude as those seen after MA administration.

We propose that MA (100 mg/kg dose) causes massive release of DA from the cytoplasmic bound pool, and inhibits monoamine oxidase (MAO). Under conditions of increased release accompanied by MAO inhibition, non-enzymatic formation of trihydroxyphenethylamines is favored. The 6-OHDA thus formed at the synapse can act like exogenously administered 6-OHDA by being taken up into the cell thereby causing neuronal degeneration. The fact that external administration of 6-OHDA as well as formed 6-OHDA are of the same order of magnitude adds further weight to the argument that the formed 6-OHDA seen after MA administration is responsible for the degeneration observed in DA terminals after MA administration.

We are currently investigating the effects of alpha-methyltyrosine administration to determine whether 6-OHDA formation can be blocked. Supported by PHS-5 PO1 DA-00250, Project IV: L.S. is the recipient of an RSA MH-10562.

346.4 EFFECT OF SUBACUTE LOW DOSE SOMAN EXPOSURE ON THE BEHAVIORAL EFFECTS OF ATROPINE. H.E. Modrow*, J.H. McDonough, and M.Z. Mays*. (SPON. B.A. Donzanti) U.S. Army Medical Research Institute of Chemical Defense, APG, MD 21010.

We have previously reported that chronic exposure to the organophosphate compound, soman, leads to a reduction in blood and brain cholinesterase activity and the number of brain muscarinic receptors. Others have reported that such a reduction in receptors should be manifested by an increased sensitivity to the behavioral effects of anticholinergics. The present study was designed to determine whether rats repeatedly exposed to soman developed an enhanced sensitivity to the behavioral effects of atropine.

Rats (N=27) were trained to stable performance baselines on a two component [fixed ratio (FR) 10 - extinction] operant task for milk reinforcement. An atropine (veh, 1.0, 1.8, 3.2, 5.6, 10.0 mg/kg, im.) dose effect curve was first determined in all animals. Each subject then received 35 µg/kg soman sc. 3 times/wk for 4 wk. The day after the last soman dose and at 4 and 8 wk later, groups of animals were tested with one of four previously tested doses of atropine (1.8, 3.2, 5.6 and 10.0 mg/kg, im.).

The initial atropine dose effect curve showed orderly dose related decreases in FR 10 responding for the 3.2, 5.6 and 10.0 mg/kg doses of atropine. Atropine had no effect on extinction responding during this or any phase of the experiment. The 35 µg/kg dose of soman had no disruptive effect on FR 10 or extinction responding throughout the subacute injection phase. The atropine dose effect curve for FR 10 responding showed that there was a significant, and parallel, shift to the left on the day immediately after the last dose of soman. However, the dose effect curves at 4 and 8 wks were the same as the initial curve determined before the series of soman injections.

Previous work has demonstrated that a similar regimen of soman exposure produces >80% reduction in both blood and brain cholinesterase and ~ 30% reduction in brain muscarinic receptors, and that both enzyme levels and receptor numbers approach control values 24 days after exposure. The present results show that performance was maintained at normal levels throughout the subacute exposure period despite the marked alteration in neurochemical parameters. However, one day after the subacute phase there was an enhanced sensitivity to anticholinergics probably due to the reduction in muscarinic receptors. This enhanced sensitivity was not evident 4 or 8 wks later, consistent with the recovery of receptors numbers and enzyme activity after repeated soman exposure.

346.5 DECREASED SLEEP IN RATS FOLLOWING ACUTE INJECTIONS OF DI-ISOPROPYL-FLUOROPHOSPHATE (DFP). C. Atwood, Jr.*, G. Meighen*, J. Gnadt, and G. V. Pegram. Neurosciences Program, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

We have shown rats to have increased REM sleep following chronic administration of DFP, an "irreversible" acetylcholinesterase inhibitor (Gnadt and Pegram, Soc. Neurosci. Abs. Vol. 9, 1983). To study the effects of acute dosing of rats with DFP on sleep parameters, we did the following experiment.

From female Sprague-Dawley rats, 200-300 gms, we recorded neocortical and dorsal hippocampal EEGs and either movement or EMG, following an acute i.p. injection of DFP at 1.25 mg/kg (low dose), 2.0 mg/kg, or 2.5 mg/kg (2.0 and 2.5 are both considered high doses and were combined). To one-half of the experimental rats, we also gave 3.0 mg/kg of atropine sulfate subcutaneously 30 min. before the DFP inj. to test its ability to alleviate the symptoms of DFP toxicity. All rats were given a baseline recording and then had recordings on the injection day, post-inj. day 1, and post-inj. day 3. All records lasted 7½ hours.

In each case, we saw a dose-dependent decrease in both stages of sleep, slow wave (SWS) and REM, following the acute inj. of DFP. On the injection day, the means of %SWS and %REM were significantly less than the control means. This decrease in sleep is probably due to the illness and discomfort caused by acute DFP poisoning. We furthermore found that atropine at 3.0 mg/kg (a dose which has no independent effect on sleep) did not alleviate these symptoms to the extent that the rats slept a significantly greater portion of the record, although there was a trend in that direction. Finally, the recovery time of sleep parameters in DFP-treated rats was found to be dose-dependent, showing a return to baseline levels within 3 days:

	Control		1.25 DFP		2.50 DFP	
	NA(9)	WA(6)	NA(6)	WA(6)	NA(4)	WA(6)
%SWS	41.0 ± 7.6	53.4 ± 2.3	4.9 ± 4.2 *	15.5 ± 5.6 **	0.48 ± 0.48 *	5.4 ± 5.3 **
%REM	9.5 ± 1.2	8.2 ± 1.2	0.0 ± 0.0 **	1.7 ± 1.4 *	0.18 ± 0.18 **	0.25 ± 0.25 **

values = mean ± SEM; () = N; NA = no atropine; WA = with atropine
* = $p < 0.01$; ** = $p < 0.001$

Supported by USARDC Contract DAMD 17-83-C-3040.

346.6 CHANGES IN MOLECULAR FORMS OF ACETYLCHOLINESTERASE IN RAT STRIATUM FOLLOWING HALOPERIDOL TREATMENT. S.P. Mahadik, A. Korenovsky* & S. Karpiak (SPON: W.C. Clark). Div. of Neuroscience, NYS Psychiatric Inst., Depts. Psychiatry & Biochemistry, Coll. of Phys. & Surg., Columbia U., N.Y., N.Y. 10032.

Extrapyramidal side-effects induced by chronic haloperidol treatment can be ameliorated with anticholinergic agents. This suggests that reduced striatal dopaminergic activity enhances cholinergic activity, which most probably contributes to the extrapyramidal side-effects. Haloperidol treatment reduces levels of acetylcholine substantially (1,2). This decrease could be caused by an increase in acetylcholinesterase (AChE) resulting from increased cholinergic transmission.

To test this hypothesis we have studied the levels of total AChE and its 4s and 10s molecular forms. The 10s ["functional"] form is predominantly membrane associated and enriched in synapses. The 4s ["precursor"] form is found only in low concentrations. Adult male Sprague/Dawley rats (250g) were treated for either one or three weeks with daily injections of haloperidol (2mg/kg,i.m.). Striata were dissected from drug treated rats and saline controls. Membrane AChE was extracted at 25° with buffer (10mM Naphosphate, 0.2mM EDTA, 50mM MgCl2, 320 mM sucrose, 1% Triton, pH 7.0). The true AChE was determined by inhibiting pseudoesterases with ISO-OMPA. Total AChE was determined in extracts and 10s and 4s forms were determined after separation on sucrose gradients. In rats treated for one week total AChE activity increased an average of 11%, the 10s form increased 26% and the 4s form showed no change. In animals treated for 3 weeks, total AChE increased 35%, the 10s form increased 150% and the 4s form showed no change. The increase in the 10s form supports the hypothesis that synaptic AChE increases in response to increased acetylcholine release following neuroleptic blockade of dopaminergic transmission. Since the increase in the 10s form is larger than the increase in the total AChE, we believe endogenous inhibitors may exist that are removed by sucrose gradient separation.

1. Guyenet et al., Naunyn-Schmiedebergs Arch. Exp. Path. Pharmak. 288:329 (1975).
2. Coyle & Campochiaro. J. Neurochem. 27:673 (1976).

346.7 DOSE-RESPONSE RELATIONSHIPS IN GROWTH, ANALGESIA, STARTLE, AND ACTIVITY CHANGES SEEN IN RATS TREATED NEONATALLY WITH MONOSODIUM L-GLUTAMATE. N. M. Wise* and K. R. King. Dept. of Psychology, Kenyon College, Gambier, OH 43022

Treatment of neonatal rats with monosodium L-Glutamate results in the formation of discrete brain lesions, retinal damage, and abnormalities of growth and behabior. Typical findings include stunted growth, obesity, reduced fertility, increased excitability, and tail automutilation. Recently, it has also been reported that MSG rats show increased sensitivity to pain and a reduced ability to respond to stress with analgesia.

While there have been studies examining how different doses of MSG affect cell loss, there has been little research relating dose to the various physical and behavioral changes mentioned above. It was the purpose of this study to examine the effects of different doses of MSG on a variety of measures. Doses ranged from the widely used 4 mg/gm (on alternating days over the first ten days of life) down to a single injection of 2 mg/gm on day two.

Although the results are not yet complete, some interesting finding have been noted. As expected, the most profound changes have occured in those animals receiving the higher doses, with comparatively weaker effects seen in those treated with smaller doses. Summarizing, MSG animals show: a reduction in body weight (with smaller stature, but greater adiposity), 2) an increase in activity (males only) and in rearing behavior, 3) a reduction in startle latency (females only) and startle magnitude (males only), and 4) tail automutilation (15% of highest-dose animals).

A curious pattern of results was found in the measures of analgesia. MSG rats were found to be hypoalgesic when tested with the tail-flick test, but when tested with the hot-plate (pawlick) test these same animals proved to be hyperalgesic. Since others have found hyperalgesia using other tests, it seems that the increased tail-flick latencies may simply reflect a local insensitivity of the tail. This not only explains the discrepency between the two pain tests, but also offers an explanation for the well-known tail automutilation which occurs in MSG rats.

346.8 NICOTINE BEHAVIORAL ACTIVATION AND CNS NICOTINIC BINDING INCREASE AFTER A FEW SMALL DAILY DOSES OF NICOTINE. C. Ksir, R.L. Hakan* and K.J. Kellar. Dept. of Psychology, Univ. of Wyoming, Laramie, WY 82071 and Dept. of Pharmacology, Georgetown Univ.

Rats tested in photocell activity cages and given repeated daily administrations of nicotine show increasing levels of behavioral activation (e.g. P.B.S. Clarke & R. Kumar, Br. J. Pharmac., 80, 587, 1983). This increase in the stimulant properties of nicotine has been interpreted as reflecting tolerance to a competing depressant action of nicotine. However, recent reports indicate that chronic exposure to large doses of nicotine produce an increase in the number of CNS nicotinic receptors (R.D. Schwartz & K.J. Kellar, Science, 220, 214, 1983; M.J. Marks, J.B. Burch, & A.C. Collins, J. Pharmac. exp. Ther., 226, 817, 1983). The current experiments were carried out to determine if the low doses of nicotine that produce enhanced behavioral activation when given daily would also produce increases in CNS nicotinic receptor numbers.

Adult male rats were adapted to photocell test cages for one hour prior to each day's test. Each rat was then given a s.c. injection of either saline or nicotine sulfate (0.1, 0.2 or 0.4 mg/kg) and replaced in the test cage. Alternately breaking the front and rear photocell beam in each cage recorded a "cage crossing". Each rat received the same drug each day for five days. Six rats were given saline, six were given 0.1 mg/kg, five were given 0.2 mg/kg, and six were given 0.4 mg/kg nicotine. Five or six hours after the last test session each rat was killed by decapitation, and its brain was quickly removed and frozen on dry ice. The brains were later thawed, dissected, and assayed for acetylcholine binding to nicotinic receptors according to the method of Schwartz and Kellar (Molec. Pharmac., 22, 56, 1982).

Cage crossings in the first ten minutes after injection increased over days in the nicotine groups, while remaining relatively stable in the saline group. By the fifth day there was a clear dose-related stimulant effect of nicotine. A mixed-design ANOVA found significant effects of group, days, and group x days interaction (all $ps < 0.001$). The three nicotine groups were all found to have significantly increased numbers of nicotinic binding sites in the cerebral cortex (+21% for the 0.1 mg/kg group, +18% for the 0.2 mg/kg group, and +26% for the 0.4 mg/kg group).

These results demonstrate that even a few daily injections of low doses of nicotine can result in upregulation of nicotinic receptors in the cerebral cortex. This upregulation may be responsible for the enhanced behavioral activation produced by the same doses of nicotine.

346.9 PHARMACOLOGY OF HALOPERIDOL-INDUCED ORAL MOVEMENTS IN RATS: A MODEL OF TARDIVE DYSKINESIA. William W. Sant III, Dept. Psychol., UCLA, L.A., CA 90024.

We and others have demonstrated increased oral movements in rats during and/or after various durations of chronic neuroleptic administration. It has been proposed that these neuroleptic-induced oral movements may be homologous to human tardive dyskinesia (TD). If this is true then these oral movements should be suppressed by an augmentation of neuroleptic dosage and, according to most investigators, should be exacerbated by antiparkinsonian anticholinergics, such as trihexyphenidyl. Thus, we examined the effects of haloperidol (0.05 mg/kg) and trihexyphenidyl (2 mg/kg) on chronic haloperidol-induced oral movements during haloperidol administration.

Twenty, female, Sprague-Dawley rats with a mean body weight of 286 gm were divided into two groups. One group received haloperidol and lactic acid in their drinking water (0.0105 mg haloperidol/ml) for 49 weeks, while the other group received lactic acid alone. At various times during and following haloperidol administration the frequency and duration of the following oral movements were scored: oral movements alone (OM), and tremor of the masseter region with or without accompanying oral movements (TRO).

In animals receiving haloperidol chronically, OM duration but not TRO duration, was elevated 39 weeks after initiation of drug administration but not before. Trihexyphenidyl reduced OM duration and TRO duration in haloperidol animals, but had no effect in controls. Haloperidol injection caused a marked decrease in OM duration, but not TRO duration, in animals receiving haloperidol chronically, whereas these measures were unaffected in control rats.

Some of these results support the hypothesis that chronic neuroleptic-induced oral movements in rats are homologous to human TD. First, OM duration was not elevated until 39 weeks after the initiation of haloperidol administration. Second, OM duration was suppressed by administration of additional haloperidol. The fact that the elevation of OM duration was abolished by acute trihexyphenidyl administration runs counter to the conventional notion of the effects of anticholinergics on TD. However, there are a number of reported cases of human TD where the symptoms of TD were potently suppressed by anticholinergics.

These results support the hypothesis that chronic neuroleptic-induced oral movements in rats are at least partially homologous to human TD.

346.10 EFFECTS OF CHRONIC NALTREXONE ON OPIATE BINDING IN DOPAMINERGIC BRAIN REGIONS AND ON OPIATE REINFORCEMENT. M.T. Bardo and J.L. Neisewander. Dept. of Psychology, University of Kentucky, Lexington, KY 40506.

Chronic exposure to opiate antagonists produces a compensatory increase in opiate receptors in the brain. Since opiate receptors are thought to have a modulatory role in the neurotransmission of dopamine systems, chronic opiate receptor antagonism might be expected potentiate morphine-induced behaviors that are mediated by dopamine. Recent evidence indicates that opiate reinforcement involves a dopaminergic substrate. The present study therefore examined the effects of chronic exposure to the opiate antagonist naltrexone on opiate binding in major dopaminergic regions in brain and on morphine reinforcement.

Adult male Sprague-Dawley rats were implanted subcutaneously for 10 days with a slow-release pellet of naltrexone (10 mg free base). The pellet was then removed and one day later the animals were decapitated. Control animals were treated similarly, except no pellet was implanted. Brains were dissected into substantia nigra/ventral tegmentum, striatum, olfactory tubercle, and medial prefrontal cortex. Each tissue region was assayed for specific binding of .66nM ^{3}H-naloxone.

In an independent experiment, rats were implanted with a naltrexone pellet and tested for morphine reinforcement either with the pellet intact or removed. Control animals were treated similarly, except no pellet was implanted. The reinforcing efficacy of morphine (5 mg/kg) was assessed using the conditioned place preference procedure.

Specific binding of .66nM ^{3}H-naloxone in control animals, expressed as fmol/mg wet weight, was 3.1 in substantia nigra/ventral tegmentum, 6.0 in striatum, 2.9 in olfactory tubercle, and 3.3 in medial prefrontal cortex. Chronic naltrexone treatment increased specific ^{3}H-naloxone binding by 52% in substantia nigra/ventral tegmentum, 54% in striatum, 28% in olfactory tubercle, and 27% in medial prefrontal cortex. Despite this increase of opiate binding in dopaminergic regions, chronic naltrexone treatment did not alter significantly the conditioned place preference observed with 5 mg/kg morphine. Nonetheless, conditioned place preference to morphine was blocked completely in animals tested with an intact pellet. The present results suggest that opiate reinforcement assessed by conditioned place preference may not be subject to supersensitization.

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346.11 BEHAVIORAL EFFECTS OF SCOPOLAMINE AND LITHIUM ADMINISTRATION IN RATS: ROLE OF THE AREA POSTREMA. K.-P. Ossenkopp, C. Sutherland* and R. L. Ladowsky. Dept. Psychology, University of Western Ontario, London, Ontario, Canada, N6A 5C2.

The area postrema (AP), a circumventricular organ in the fourth ventricle, has been shown to be a chemoreceptor for blood borne toxins (Borison, Life Sci., 1974, 14, 1807). Several studies have shown that AP is the central chemoreceptor for injected toxins used to establish conditioned taste aversions (CTA) in animals without an emetic reflex, such as rats. AP ablation has been shown to abolish CTAs induced by injections of methylscopolamine and LiCl and we wanted to know if other behavioral effects produced by these drugs might also be mediated by AP.

Subjects were adult male hooded rats kept on a 23 hr/day water deprivation schedule. In Experiment 1 rats were given thermal cautery lesions of AP or sham lesions. Following a 2 week recovery period both groups of animals were injected with either 1 mg/kg (i.p.) scopolamine methyl nitrate (SMN) or 1 mg/kg (i.p.) scopolamine hydrochloride (SHC). Pairing of these drug injections with a saccharin taste resulted in strong CTAs in sham lesioned rats but not in AP lesioned animals. Both AP and sham lesioned rats exhibited comparable hyperactivity following SHC and both groups showed small reductions in activity following SMN injections. In Experiment 2 AP and sham lesioned rats were tested for CTA formation when LiCl (0.15M, 10 ml/kg) was administered by i.p. injection or by intragastric intubation. Sham lesioned rats acquired strong CTAs with both routes of administration. AP lesioned animals failed to form any CTAs. In Experiment 3 AP and sham lesioned rats were familiarized with the ingestive effects of 0.15M LiCl and NaCl. On the test days the rats were given access to either LiCl or NaCl for 1 hr. When given LiCl the AP lesioned rats drank significantly more than the sham lesioned animals. Following LiCl ingestion the sham lesioned group exhibited significant decrement in rearing activity whereas the AP lesioned group did not. There were no significant differences in intake and rearing during access to NaCl between the groups. Thus, AP mediated CTAs induced by i.p. injections of SMN, SHC and LiCl, and by intragastric LiCl. AP also mediated LiCl induced hypoactivity but not SHC induced hyperactivity.

(Supported by Natural Sciences and Engineering Research Council grant U0151 to K.-P. Ossenkopp)

346.12 THE EFFECTS OF PRENATAL INJECTIONS OF METHYLAZOXYMETHANOL (MAM) ON REARING BEHAVIOR IN DEVELOPING RATS. M.A. Henault, P.R. Sanberg, T.H. Moran, and J.T. Coyle. Behavioral Neuroscience Laboratory, Dept. of Psychology, Ohio Univ., Athens, OH 45701 and Depts. of Neuroscience & Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Pregnant rats which received injections of the antimitotic agent MAM on day 15 of gestation (E15) produce offspring which have marked hypoplasia of the telencephalon and a hyperinnervation of ascending neurotransmitter systems to the forebrain (Johnston & Coyle, J. Neurochem. 34:1429, 1980). The offspring of MAM injected rats display a number of behavioral deficits including decreased behavioral organization following stimulation of the MFB, decreased apomorphine stereotypy, and hyperkinesis. The present study extended these findings by investigating the effects of MAM on open-field rearing behavior in developing rats.

Pregnant rats were injected i.p. on E15 with either 20 mg/kg of MAM or saline. The offspring were then divided into one of four groups: MAM males, MAM females, Control males, Control females. At 10,15,20,25, or 30 days of age, rats were placed into computerized Animal Activity Monitors (Omnitech, Inc.) for 15 minutes. During this time various measures of vertical activity (rearing behavior) were analyzed by the Digiscan computer. In addition, an observer counted the number of rears. Statistical differences between groups were analyzed using ANOVA techniques.

The results indicated that rearing behavior appeared around Day 15 and then increased in an almost linear fashion through Day 30, with a slight decrease at Day 25. Furthermore, the total time spent rearing and the average duration of each rear increased from Day 10 through Day 30. Although there were no significant differences between drug groups, MAM rats tended to rear more than controls through Day 25, whereas at Day 30 this trend was reversed. Across age, MAM rats spent more total time rearing and reared for shorter durations than controls. Finally, differences between sexes were found in both the total time and the average duration of the rearing response.

These findings support a role for the telencephalon in the mediation of the rearing response. However, the moderate deficits seen in MAM rats may indicate compensation of function due to neuroplastic events within the lesioned telencephalon, and/or the importance of other neural systems in the control of rearing behavior. Supported by MH26654, the Pratt Family and Friends HD grant, OURC, and the Tourette Syndrome Association.

346.13 BEHAVIORAL EVALUATION OF PERINATAL PCB EXPOSURE IN RHESUS MONKEYS: FIXED-INTERVAL PERFORMANCE AND REINFORCEMENT OMISSION. P.C. Mele* and R.E. Bowman. SPON: V. DeNoble. Primate Lab., Univ. of Wisconsin, Madison, WI 53706

Perinatal exposure to polychlorinated biphenyls (AROCLOR 1248) altered locomotor activity of rhesus monkeys for up to 4 yrs. of age. Relative to controls, monkeys whose mothers were fed 0.5 ppm PCBs throughout gestation and nursing (concurrent exposure) showed locomotor hyperactivity when tested at 12 mo. of age. Monkeys whose mothers which had been taken off a 2.5 ppm diet (post exposure) showed locomotor hyperactivity at 12, 36 and 48 mo. of age.

Further evaluation of these monkeys began at approximately 42 mo. of age. Monkeys were tested under a series of fixed-interval schedules of food reinforcement: FI 30 sec (10 sessions); FI 60, 300 and 600 sec (15 sessions each); FI 60 (30 sessions). Environmental challenge was conducted with a reinforcement-omission phase during which 25% of the scheduled reinforcers were randomly omitted (10 sessions). Performance measures included overall response rate (RR), index of curvature (IC) and post reinforcement pause (PRP). RR and PRP did not differ between groups. Under FI 300 and 600 IC was slightly though consistently lower for PCB than control monkeys. The transition from FI 30 to 60 produced greater variability in RR and IC of the 0.5 ppm than the control group. During reinforcement omission sessions there were no between group differences for FIs following reinforcement delivery. For FIs following reinforcement omission the 2.5 ppm group had a significantly higher RR and greater interanimal variability in RR than the control group; RR increases following reinforcement omission were correlated ($r=.98$, $p<.02$) with locomotor hyperactivity measured at 4 yrs. of age. These data indicate altered reactivity in PCB-exposed monkeys.

346.14 LINDANE POTENTIATES HIPPOCAMPAL POTENTIALS EVOKED BY STIMULATION OF THE PREPYRIFORM CORTEX (PPC). L. Zimmer* and D. Woolley* (SPON: R. Dagirmanjian). Dept. of Animal Physiol., Univ. of Calif., Davis, CA 95616.

Lindane (γ-hexachlorocyclohexane) is a topical pesticide presently used in both human and veterinary medicine. It is also a potent convulsant agent, whose mechanisms of action are under active investigation. Because the convulsant effects of lindane may be due to an action on the limbic system, we used the evoked potential (EP) technique to determine its effects on 3 limbic pathways. The effects of a single dose (30 mg/kg per os) were studied in rats with chronically implanted electrodes. Only minimal seizure activity, primarily myoclonic whole body jerks, was observed and was maximal about 1 hr after dosing. EPs elicited in the dentate gyrus (DG) by stimulation of the PPC were potentiated as soon as 1 hr and for as long as 14 days after lindane. Maximal potentiation averaged two-fold and occurred at either 12 or 24 hrs, after which amplitudes gradually declined. The EP was potentiated even in those rats in which seizures did not occur. During the pretreatment period, paired pulse stimulation with an interstimulus interval (ISI) of 40 msec produced marked potentiation. After lindane, when both the first and second responses were potentiated, population spikes (PSs) appeared in the second response in 75% of the animals, whereas PSs never appeared during the control period. We also studied the effects of lindane on EPs elicited in the DG by stimulation of the dorsal perforant path (PP), because the PP is probably the final link between PPC and DG, and on EPs evoked in hippocampal subfield CA3 by stimulation of the DG. In both systems, the slow EPs were not affected by lindane. However, small but significant increases in the amplitude of PSs were observed 12 hrs after lindane. In addition, a second PS appeared in the CA3 EP. The increase in PS amplitude and the appearance of an additional PS could have resulted from reduced inhibition. However, recurrent inhibition in these 2 systems, tested by the paired pulse method with an ISI of 20 msec, was not affected by lindane. Thus, of the three pathways tested, the only slow EP which was potentiated by lindane was that elicited in the DG by PPC stimulation. The relatively long-term potentiation of the PPC-evoked DG response is very similar to that produced by dieldrin, as reported previously by this laboratory, and demonstrates again the marked propensity for this pathway to potentiate. (Supported by NIH grant ES-01503 to DW and fellowship number ES-05145 to LZ.)

346.15 TRIMETHYLTIN (TMT) SELECTIVELY POTENTIATES HIPPOCAMPAL POTENTIALS EVOKED BY STIMULATION OF THE PREPYRIFORM CORTEX (PPC). D. Woolley*, L. Zimmer* and Z. Hasan (SPON: V. Vijayan). Dept. of Animal Physiol., Univ. of Calif., Davis, CA 95616.

TMT is a highly toxic compound which produces some signs of poisoning characteristic of limbic system dysfunction. To determine which limbic pathways are affected by TMT, the averaged evoked potential (AEP) technique was used. The effects of a single dose of TMT chloride (7.5 mg/kg per os) on AEPs were studied in 30 rats bearing chronically implanted electrodes. This dose produced little or no seizure activity, but did produce tremors. Of the 6 pathways studied, only the EP elicited in the dentate gyrus (DG) by stimulation of the PPC was potentiated after TMT. Potentiation was maximal 5 days after TMT, which also was about the time that tin reached peak levels in brain. After the time of peak potentiation, amplitudes of the PPC-evoked DG response declined so that by 15 days after TMT values were only 48% of control amplitudes. Surprisingly, the DG response elicited by stimulation of the perforant path (PP) was not potentiated at any time after TMT, even though the PP is believed to represent the final link in the path from PPC to DG. Five days after TMT, when the PPC-evoked DG response was maximally potentiated, the PP-evoked DG response was only 65% of pretreatment values. Furthermore, the response evoked in the PPC by stimulation of the lateral olfactory tract was not significantly altered during 20 days after TMT. Responses evoked in the CA3 subfield of the hippocampus by stimulation of either DG or the commissural pathway markedly declined after TMT, whereas amplitude of the antidromic response in CA3 evoked by stimulation of the lateral septum (LS) declined only 20% a few days after TMT and then recovered. Recurrent inhibition was reduced in the DG during paired pulse stimulation of the PP a few days after TMT. Taken together, the findings indicate that reduced inhibition may have contributed to the TMT-induced potentiation of the PPC-evoked DG response. Parallel neuropathological studies indicate that the decline in amplitudes of the AEPs was due to neuronal necrosis. The selective potentiation by TMT of only the PPC-evoked DG response supports our previous reports that this system is particularly plastic and highly susceptible to long-lasting potentiation by certain toxic agents. (Supported by NIH grant ES-01503 to DW, fellowship ES-05145 to LZ, and a fellowship from Jordan to ZH.)

346.16 CHRONIC MANGANESE INTAKE INDUCES CHANGES IN THE MOTOR ACTIVITY OF RATS. E. Bonilla. INBIOMED-FUNDACITE and Instituto de Investigaciones Clínicas. Apartado 376. Maracaibo, Venezuela.

Chronic manganese poisoning has been found to induce important changes in the brain content of some biogenic amines and the activities of their synthesizing enzymes. Dopamine (DA) is involved in central regulation of motor activity. Brain L-tyrosine hydroxylase activity and DA concentrations increase in the first 3 months of manganese intake. However, continued manganese administration produced a marked decrease in the concentrations of DA and in the activity of L-tyrosine hydroxylase. We investigated modifications in the behavior of adult rats when exposed to different concentrations of manganese in the drinking water.

Experiments were carried out on male Sprague-Dawley rats weighing 150 to 250 g and fed ad libitum with rat laboratory chow (Protinal-Maracaibo). They were placed on solutions of manganse chloride (0.1 and 5.0 mg Mn^{2+}/ml) in their drinking water. Control rats received distilled and demineralized water. Spontaneous motor activity was measured using an Opto-Varimex-Minor unit (Columbus Instruments, Columbus, Ohio). A Printing Counter Model 800 (Columbus Instruments) was hooked up to the Opto-Varimex-Minor so that activity records were obtained automatically.

Spontaneous motor activity, measured in 60-min weekly sessions during 8 months, showed a significant increase during the 1st. month. Further exposure did not affect the motor activity to 6 months. But, on months 7 and 8 a significant reduction was observed compared with controls. Both hyper-and hypoactivity were not dose-dependent as the results obtained in both groups of manganese-exposed rats were similar. These findings should make health authorities aware of the potential involvement of low doses of manganese in the development of early behavioral problems long before the irreversible neurologic damage is established.

346.17 THE LONG-TERM COGNITIVE EFFECTS OF NEONATAL LEAD EXPOSURE IN MONKEYS. E. D. Levin*and R. E. Bowman. Psychology Primate Lab., Univ. of Wisconsin, Madison, WI 53715

Two cohorts of rhesus monkeys were exposed to chronic, moderate levels of lead during the first year after birth. They were tested as adults on delayed spatial alternation (DSA), a spatial memory test. The first cohort contained 3 controls, 3 low-lead, and 3 high-lead monkeys. The low and high lead groups respectively had been given 0.29 & 0.88 mg/kg/day lead acetate orally for the first year after birth. The second cohort contained 8 controls and 8 lead-treated monkeys. The lead-treated group received 1.0 mg/kg/day of lead acetate orally for the first year after birth.

The monkeys in the first cohort were tested when they were about nine years of age and the monkeys in the second cohort were tested when they were about five years of age. The blood lead concentrations in the lead groups of both cohorts had returned to normal long before the present testing. The DSA testing took place in a Wisconsin General Testing Apparatus using two red wooden squares as stimuli and chocolate bits, froot loops and M&Ms as reinforcements. The position of the reward was alternated between the right and left hand response sites on successive trials. Delays of 5, 10, 20 or 40 seconds were imposed between trials. Eight instances of each delay were used in a session. Blocks of eight sessions were used for the analysis of acquisition. Neither cohort showed lead-related differences on percent correct over the course of acquisition at any of the delays. The data are also being analyzed for response strategies and longest trains of correct and incorrect trials. These monkeys are undergoing a series of DSA sessions under the influence of agonists and antagonists of the dopaminergic and cholingeric systems to determine if these drug stresses would uncover a more subtle effect of lead. DSA has been found in our lab to show long-term effects to early life exposure to higher levels of lead. It also has shown long-term effects to early life exposure to low level polychlorinated biphenyls. On the other hand, the monkeys in the present cohorts do not appear to show a similar degree of impairment.

The monkeys in this experiment were treated in accordance with the humane practices prescribed by the Society of Neurosciences. This research was supported by NIEHS grant # ESO-1062.

346.18 FLAVOR AVERSIONS INDUCED BY LEAD ARE ATTENUATED BY CHELATORS. D.B. Peele,* R.C. MacPhail,* J.D. Farmer* (SPON: L. Reiter). Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

A series of experiments assessed the flavor aversion induced by lead acetate alone or in combination with one of two metal chelators in an attempt to antagonize the aversive properties of lead. The first three experiments assessed the flavor aversion induced by lead acetate (62.5-500 mg/kg) and two metal chelators, dimercaprol (BAL, 6-48 mg/kg) and dimercaptosuccinic acid (DMSA, 25-200 mg/kg). After intakes had stabilized on a restricted water-availability schedule (30 min/day), male Long-Evans rats (6/group) were given 30-min access to a 0.1% saccharin solution followed 20 min later by either p.o. administration of lead or i.p. administration of BAL or DMSA. Preference for water vs saccharin solution was assessed 72 hr later by presenting both fluids and measuring intake. Lead acetate and both metal chelators produced dose-related flavor aversions (i.e., suppression of relative saccharin intake); the ED50s for lead, BAL and DMSA were approximately 100, 24, and 100 mg/kg, respectively.

The fourth experiment employed a similar design in which rats (9/group) were given either lead (125 mg/kg) alone or in combination with either BAL (6-24 mg/kg) or DMSA (25-100 mg/kg) following saccharin consumption. As in the first experiment, rats receiving lead alone avoided saccharin while those receiving the sodium acetate vehicle preferred saccharin. Rats receiving both lead acetate and either of the chelators showed intermediate preference scores. An analysis of variance revealed a significant attenuation of the lead-induced aversions by both chelators. Total fluid consumption on the test day did not vary systematically between treatment groups. This attenuation of a lead-induced flavor aversion demonstrates the utility of flavor-aversion conditioning in assessing the interaction of heavy metals and heavy-metal chelators.

346.19 PRO- AND ANTI-CONVULSANT EFFECTS OF HEXACHLOROCYCLOHEXANE ISOMERS IN RATS GIVEN MAXIMAL ELECTROSHOCK. V. Stein*, R.S. Narang*, L. Wilson*, R.A. Waniewski and W. Shain, (Spon: T. Galeno) NYS Dept. of Health, Ctr. for Labs & Res., Albany, NY 12201

Gamma-hexachlorocyclohexane (HCH)--lindane is an insecticide that has convulsant properties in man. During its synthesis a number of other non-insecticidal isomers are made. HCH isomers differ by the chair conformation of the hexane ring and the axial vs. equatorial positions of the Cl^- atoms. These studies were designed to test the effects of 4 HCH isomers on maximal electroconvulsive shock (MES) induced seizures. Rats were injected with various concentrations of alpha, beta, delta and gamma HCH isomer in dimethylsulfoxide (DMSO). Initial experiments were performed to establish the overt toxicity (LD-50) and behavioral effects of the isomers. The gamma and delta isomers were found to have time and dose dependent effects on the behavioral response of the animals. Gamma-HCH was a powerful convulsant (LD-50=15 mg/kg, EC-50=8 mg/kg). Delta-HCH caused hyperexcitation (LD-50=150 mg/kg, EC-50=30 mg/kg). The other isomers caused no stereotypic changes in behavior. Since convulsant and behavioral changes were observed as early as 2.5 min animals were tested at 2 min after injection and again at 1,6,24 and 72 hrs. Animals were shocked and five elements of seizure were observed: forelimb flexion, hindlimb flexion, exten- sion, duration of total tonus, and recovery time. At one hour the gamma isomer significantly increased total tonus. The alpha isomer increased total tonus and recovery time at 6 hr. The beta isomer had no significant effect. The delta isomer significantly reduced total tonus at 2 min. The delta isomer also caused a significant increase in total tonus at 1 hr. To establish brain concentrations of HCH, animals were injected as described for behavioral studies and brain and liver samples taken for analysis. HCH isomers were separated using gas chromatography and measured with an electrochemical detector. For alpha, delta, and gamma isomers the highest concentrations were observed at 2 min after injection in both liver and brain. Liver content was higher than brain until 72 hr when both tissues had nearly equal concentra- tions. While the concentration of the beta isomer was similar to the others at 2 min this isomer continued to be concentrated over the 72 hr period of study. The rapid effects of the HCH isomers and their relative anti- and pro-convulsant properties observed in this study may be a result of rapid distribution in DMSO.

346.20 EFFECTS OF SINGLE AND REPEATED DOSES OF TOXOCARA CANIS IN MICE. L. J. Draski*, J. Baek*, R. G. Burright*, and P. J. Donovick, Dept. of Psychology and Center for Neurobehavioral Sci., SUNY at Binghamton, NY 13901, and R. H. Cypess*, Dept. of Prev. Med., Cornell Univ., Ithaca, NY 14850.

The common parasitic roundworm, Toxocara canis, completes its life cycle in the dog. The eggs of this parasite are passed by the dog's feces into the environment and may be ingested by a variety of mammals. In abberant hosts, such as mice and humans, larvae migrate through the visceral organs and the central nervous system where they may remain viable for years. The fact that there is no known cure for this infection is complicated further when one considers the world wide distribution of domestic dogs and the long-term viability of T. canis eggs in the environment.

Previously we reported that infection altered motor activity, passive avoidance performance, and sensory reactivity of mice. In the present experiment, we examined the behavioral effects of T. canis as a function of temporal distribution of infection. Intubations occurred bi-weekly until mice received four doses of T. canis eggs and/or saline in one of the following orders: 1) 1000 eggs initially followed by 3 saline intubations; 2) saline 3 times and then 1000 eggs 3) 250 eggs each time; 4) saline only. Approximately ten weeks after the first intubation, activity was measured in an open field before and after a one minute cold water swim. In the first open field, horizontal exploration (square crosses) was not statistically different among groups, but vertical exploration (stand-ups) was severely reduced in the T. canis group infected by a single dose of 1000 eggs at the onset of the experiment. The repeated dosage group and the group receiving 1000 eggs as a final dose were not differentially effected relative to controls. When tested in the open field 16 minutes after a stressful one minute swim, all groups showed a reduced number of crosses and stand-ups. However, both single-dose Toxocara groups crossed fewer squares than controls. In contrast, the repeatedly infected group was not inhibited on this measure of horizontal exploration when compared to controls. However, measures of vertical exploration were decreased in all T. canis groups.

These data suggest that at least some effects of T. canis may be diminished as a function of prior exposure to this parasite. This "sensitization" effect may have important public health implications, particularly for children with known pica for dirt and where repeated exposure is the most likely route of infection.

346.21 INCREASED SUSCEPTIBILITY TO CHEMICALLY-INDUCED SEIZURES AFTER EXPOSURE TO ORGANIC LEAD. H.S. Swartzwelder. Duke University and V.A. Medical Centers, Durham, N.C. 27705.

Organic leads are environmentally ubiquitous due to their primary use as fuel additives. Despite extensive production of organic lead for this purpose, little is known about the possible toxic effects of these compounds. Although the tetraalkyl leads are metabolized quickly in biological systems, their trialkyl metabolites persist for considerably longer periods. In fact triethyl lead (TEL) has a half-life of up to eight days in brain. Thus the trialkyl leads may pose a realistic environmental threat, and further this threat may be a neurotoxic one.

Previously we have used the susceptibility to seizures as an index of general neurotoxicity induced by a variety of putative neurotoxins. In the present study the responsiveness of rats to a challenge dose of pentylenetetrazole (PTZ) was assessed after exposure to TEL.

Seventy-two male rats of the Fischer-344 strain were housed on a 12:12-h light/dark cycle with continuous access to food and water throughout the experiment with the exception of PTZ challenge tests. The experiment was designed so that separate groups of 12 rats each were treated with 7.88 mg/kg/ml of TEL s.c. at 1, 7, 14 and 28 days before the single challenge with PTZ. In addition, two control groups were given injections of the saline vehicle at 1 and 28 days prior to PTZ challenge. On the appropriate days the rats were given a single injection of PTZ (35 mg/kg/2.0 ml I.P.). Immediately after the injection, the rat was placed in a sound-attenuating chamber and observed for 10 min. Each rat was assigned a seizure score on a numerical scale from 1 to 5 depending upon the severity of epileptiform response observed.

A small increase in seizure susceptibility was observed relative to controls in the rats which were tested one day after exposure to TEL. This effect was not statistically significant. However, at Days 7, 14 and 28 after exposure, the TEL-treated rats received markedly higher seizure scores than did controls. These results suggest that TEL produces a neurotoxic effect which is evident as early as one day after a single exposure. Moreover, the CNS of the TEL-treated rat appears to be compromised for at least four weeks after a single systemic dose of this organic lead.

346.22 PERSISTING ALTERATIONS IN BEHAVIOR AND BRAIN NEUROCHEMISTRY FOLLOWING CONTINUOUS LOW-LEVEL LSD. G. Ellison and W. King, Jr.* Dept. of Psychology, UCLA, Los Angeles CA 90024.

D-amphetamine administration to animals can induce a long-lasting alteration in caudate dopamine terminals suggestive of a selective and persisting neurotoxicity. This effect is particularly striking when the drug is present in brain for prolonged periods, such as when administered via slow-release pellets or in combination with drugs which inhibit amphetamine breakdown. In the present study we investigated whether comparable neurotoxic effects could be detected in brain after continuous administration of the hallucinogen LSD.

In an initial study, groups of rats were implanted with osmotic minipumps which delivered 80ug of LSD tartrate over a 7 day period, or were given 7 daily LSD injections, each of 11.4 ug, or were implanted with control pellets. When tested 30 days after termination of drug treatment, only the rats administered continuous LSD differed from the controls: they were inactive in open field tests and, when tested with social partners, showed increased huddling.

In order to determine which brain structures mediated this persisting behavioral alteration, similarly prepared rats were sacrificed 30 days after LSD treatment; regional HPLC assays of 5HT, 5HIAA, DA, DOPAC, and HVA were made in Frontal cortex, Hippocampus, Caudate, and Nuc. Raphe. No consistent differences were found except in Hippocampus, where the minipump animals had decreased 5HT levels.

In a third study, similarly prepared animals were studied 30 days after drug cessation using autoradiography of common ligands. Frozen brain sections were incubated in tritiated spiroperidol, 5HT, or LSD, washed, dried, and stored against Ultrafilm plates. When developed and quantified using an optical densitometer, large increases in LSD binding in only the minipump animals were found in a variety of forebrain structures (Frontal cortex, amygdala, septum, nucleus accumbens) but not in lateral geniculate, hypothalamus, substantia nigra, visual cortex, and other brain regions.

Continuously administered LSD induces long-lasting changes in behavior and in forebrain binding of LSD, whereas the same amount of LSD administered in daily injections does not. The exact mechanisms underlying this effect remain to be determined. We hypothesize that LSD is potent in inducing long-lasting alterations in humans in part merely because of its prolonged action.

346.23 IMPAIRED ACQUISITION OF A DELAYED REINFORCEMENT AUTOSHAPED BEHAVIOR AND REDUCED MUSCARINIC CHOLINERGIC LIGAND BINDING IN HIPPOCAMPI OF RATS TREATED WITH TRIMETHYLTIN. C.A.Cohen*, R.B.Messing, and S.B. Sparber*. Dept. of Pharmacology, Medical School, University of Minnesota, Minneapolis, MN55455

Deficits in maze learning and hippocampal lesions have been reported following injection of 5.0-7.5 mg TMT/kg (Dyer et al., Neurobeh.Tox.Terat. 1982,4:141;Swartzwelder et al., ibid,169; Walsh et al., ibid ,177), to rats. Weight loss and other signs of toxicity are associated with high doses.

An experiment with 3.0 and 6.0 mg TMT/kg p.o. resulted in dose-dependent reductions in the hippocampal binding of the muscarinic antagonist ^{3}H-QNB 5 months after an injection of the organotin. However, no evidence of altered acquisition or performance of autoshaped lever touch behavior maintained by food reinforcement was evident prior to decapitation for ^{3}H-QNB binding studies. A more difficult autoshaped task, in which a 6 second delay was interposed between retraction of the lever and the delivery of the food pellet (noncontingent or contingent upon a touch response) was used in the second experiment. The lever was inserted into the chamber on a 45 second random time schedule , and was withdrawn either when the animal touched it, or noncontingently after 15 seconds. Rats were given saline or TMT (3,6 or 7.5 mg/kg). Ten days later, and during partial food deprivation, 23 days later, no effects of TMT on motor activity were found in the same chambers later used for autoshaping (Lichtblau and Sparber,ibid,557). As expected, body weight loss, convulsions, and irritability were associated with injection of 7.5 mg TMT/kg, but overt signs of toxicity were absent at lower doses. Thirty days after treatment, animals were exposed to a single 12 trial autoshaping session; session 2 was run 5 days later, and sessions 3-7 were run daily thereafter. The highest dose of TMT (7.5 mg/kg) did not significantly impair autoshaped responding of the group relative to controls, but performance was characterized as more variable, rats that convulsed tending to acquire the response. The two lower doses caused a dose dependent retardation (3.0 mg/kg) or blockade (6.0 mg/kg) of acquisition. Experiments are being conducted to ascertain the nature of the apparent U-shaped function of the dose-response curve for TMT upon delayed reinforcement behavior. Supported by USPHS T32DA07097, K07ES00123, Minnesota Medical Foundation.

346.24 SEX DIFFERENCES IN THE RESPONSE TO ANTIDEPRESSANT TREATMENTS Ellen Stotsky*, Mali Israeli* and Anat Biegon, Weizmann Institute of Science, Rehovot, Israel.

Clinical studies have shown that females have a higher incidence of depression than males while they benefit less from antidepressant drug therapy. Electroconvulsive shock (ECS) appears to be equally effective in both sexes. We have studied the effect of two weeks administration of Amitriptyline (Ami, 10 mg/kg/day) or 10 days of ECS (100v/animal/day, through ear clips) on β adrenergic and S_2-serotonergic receptors in frontal cortices from male and female rats, in an attempt to find a possible neurochemical correlate for these observations. We find that female rats have significantly higher levels of cortical β adrenergic receptors than males. Bmax using scatchard analysis of 3H-DHA binding data was 94±11.1 fmoles/mg protein for control females and 75±6.5 fmoles/mg p. in males, $p<0.05$, mean of 7 experiments. However, the females did not respond to Ami by a significant reduction in β adrenergic receptor number, while the expected decrease (16%, significant at $p<0.05$) was observed in the males. S_2 receptor number in the males decreased considerably following Ami (30%, $p<0.01$). Again, there was no effect on the females.

ECS resulted in a significant decrease in the Bmax of β adrenergic receptors in both sexes (20% reduction, significant at $p<0.02$). S_2 receptors which were significantly increased in the males following ECS (36%, $p<0.05$) were not significantly altered in the females. Thus it appears that cortical β adrenergic receptors in female rats are resistant to the induction of subsensitivity by Ami but not by ECS. S_2 receptors in females are not changed by either treatment. If such a trend exists also in humans, it may contribute to the poorer response of female patients to tricyclic antidepressants.

OTHER DRUGS OF ABUSE

347.1 PHENCYCLIDINE (PCP) BINDING IN THE NUCLEUS ACCUMBENS (NAc) AND PCP-INDUCED HYPERACTIVITY ARE DECREASED FOLLOWING 6-HYDROXYDOPAMINE (6-OHDA)LESIONS OF THE MESOLIMBIC DOPAMINE SYSTEM. E.French, G.Vantini*,P.Contreras*,C.Pillapil and R.Quirion. Maryland Psychiatric Res.Ctr.,Baltimore,MD 21228, NINCDS,Bethesda,MD 20205 and Douglas Hospital Res.Ctr. Verdun, Canada.

Recently we published that 6-OHDA lesions of the NAc blocked PCP and amphetamine but not caffeine or scopolamine-induced hyperactivity. Thus, it was concluded that PCP elicits locomotor hyperactivity through a DA presynaptic mechanism in the NAc. Since specific high affinity PCP binding sites have been shown to exist in the NAc, the present study was designed to evaluate the possibility that destruction of DA mesolimbic terminals would also be accompanied by a loss of PCP binding.

Rats were anesthetized and injected bilaterally with 6OHDA (4ug/ul) into the ventral tegmental area(VTA)(1ul) or NAc(2ul) and sacrificed 21-28 d later. ^{3}(H)PCP binding was measured in the NAc, striatum and hippocampus as described elsewhere (in submission).

Following 6-OHDA injections into the VTA there was a significant decrease in the number of NAc PCP binding sites (421 fmoles/mg prot) compared to vehicle injected rats (773 fmoles/mg prot). 6-OHDA injected directly into the NAc produced an even greater loss(74%) of PCP binding (controls: 872 vs lesion:224 fmoles/mg prot). Although the K_d values were also higher in lesioned rats the exact significance of this observation remains to be determined. Striatal and hippocampal PCP binding was not significantly changed following VTA or NAc 6-OHDA. Both lesioned groups also showed a highly significant depletion of NAc DA content. An additional group of rats(n=5) was used for both PCP binding analysis and assessment of locomotor responsiveness (photocell equipped cages) to PCP(5mg/kg),caffeine(10mg/kg) and scopolamine(1.5 mg/kg) following 6-OHDA lesion of the VTA. Again, compared to controls PCP binding sites were found to be significantly reduced as was PCP-induced hyperactivity. In contrast, caffeine and scopolamine still elicited hyperactive behavior. In a more recent experiment, locomotor measures were made in a group of rats 14-22 days following bilateral kainic acid injection(1ug/ul) into the NAc. All 3 stimulant drugs produced hyperactivity. In these kainate lesioned rats PCP binding,DA and CAT content are now being assessed.

These data suggest that PCP elicits its locomotor stimulating effects via an interaction with PCP receptors located mostly on terminals of mesolimbic DA fibers.(supported byPMA)

347.2 AGONIST AND ANTAGONIST ACTIONS OF PHENCYCLIDINE (PCP) DERIVATIVES ON PCP RECEPTORS AND BEHAVIOR. P. C. Contreras*, M. F. Rafferty*, A. E. Jacobson*, K. C. Rice*, R. Quirion and T. L. O'Donohue, ETB, NINCDS, LC, NIADDK, NIH, Bethesda, MD 20205 and Douglas Hosp. Res. Ctr, Verdun Quebec, Canada H4H 1R3.

We correlated receptor binding and behavioral potencies of a series of PCP analogs to develop a method for testing the agonist or antagonist actions of PCP analogs and endogenous peptides (Quirion et al., in press), which bind to PCP receptors. Stereotypy and ataxia were determined at various times after intraventricular administration of PCP, PCP analogs and saline to rats using a behavioral rating scale (Sturgeon et al., Eur. J. Pharmacol. 59:169, 1979). Peak effects of PCP and a series of agonists, which bind to the PCP receptor, occurred in a dose-dependent manner 5 min after injection. The ability of PCP analogs to induce stereotypy was stereoselective as dexoxadrol and (+)PCMP were more potent than their levo isomers, levoxadrol and (-)PCMP. The fact that only levoxadrol was more potent than dexoxadrol in producing ataxia indicates that ataxia is not a good index of PCP binding. The behavioral effects were not due to interactions with mu, kappa or delta opioid receptors because naloxone pretreatment did not alter the ED50 of PCP for inducing stereotypy or ataxia. Pretreatment of rats with Metaphit (1 umol/rat), a PCP analog that alkylates PCP receptors (Rafferty et al., in press), had dramatic actions on PCP-induced behavior. One hr after Metaphit pretreatment, which alone did not produce a significant degree of stereotypy or ataxia, the ED50 of PCP for inducing stereotypy was increased 9-fold, but the ED50 of PCP for inducing ataxia was only slightly increased. Twenty-four hr after Metaphit pretreatment, the ED50 of PCP for inducing stereotypy and ataxia was increased 3- and 2-fold, respectively. Scatchard analysis of PCP binding to a crude membrane preparation obtained from the brains of rats pretreated with Metaphit or saline 24 hr before sacrifice showed a significant decrease in Bmax of treated versus control rats. In conclusion, these results indicate that stereotypy induced by PCP agonists is a good indicator of PCP receptor interactions, but ataxia is a poor indicator because ataxia is mediated by more than just an interaction with PCP receptors. Also, the ability of Metaphit to antagonize the behavioral effects of PCP suggests that it may not only be a useful tool in elucidating the mechanism of action of PCP and treating PCP-induced psychosis, but may also be a general antipsychotic.

347.3 CLINICAL AND PHARMACOKINETIC ASPECTS OF PHENCYCLIDINE (PCP) ABUSE. D.A. Gorelick, J.N. Wilkins*, G.B. Smith* and B.E. Derrick*. Psychiatry Serv. & Clin. Psychopharmacol. Unit, West LA VA Med. Center, Los Angeles, CA 90073.

Recent studies using a highly sensitive gas chromatographic assay with nitrogen/phosphorus detection (GC-N/P) have reported PCP in body fluids of half or more of unselected psychiatric patients presenting to a large, urban hospital. The clinical significance of these findings and their applicability to other patient populations remain unclear, in part because of uncertainty over the persistence of PCP in the body. In this study, urinary PCP screening was done on 155 consecutive inpatients admitted over a 9-month period to a voluntary VA substance abuse treatment program. Urines collected on admission (and weekly thereafter if PCP-positive) were assayed for PCP using GC-N/P (sensitivity = 0.1 ng/ml). Forty-two (27%) patients had PCP detected in their admission urine (mean level = 755.9 ng/ml, range = 0.13-12,028 ng/ml). Of these, 28 gave a history of PCP use, while 14 initially denied it. (In addition, 14 (9%) patients with PCP-negative urine gave a history of PCP use.) There was a significant inverse correlation between self-reported time since last PCP use and initial urine PCP level ($r = -0.48$, $df = 31$, $p < .01$). Visual inspection of the graph of these 2 variables suggested a biphasic elimination process: a more rapid initial phase with a half-life of 5-7 days, and a slower second phase with a half-life of more than 30 days. Actual PCP levels became undetectable by 4 weeks of admission in all except 5 patients suspected of PCP reuse on clinical grounds. Twenty patients (all with PCP-positive urine) met DSM-III criteria for PCP abuse. These abusers had smoked PCP for a mean of 7 years, with 7 smoking it daily. They also frequently abused other drugs: alcohol (n = 9), marijuana (7), stimulants (3), and opiates (2). PCP abusers differed from non-abusive users in having higher PCP levels at admission (mean = 1513.7 vs. 41.0 ng/ml), more recent PCP use (4.6 vs. 1290 days before admission), younger age (31.6 vs. 40.2 years), and more arrests (2 vs. 0.8). These findings indicate that detectable PCP levels may persist in body fluids for several weeks after its last use, and suggest that routine urinary screening of substance abuse patients using a sensitive PCP assay may detect a significant minority who initially deny PCP use.

(We thank the nursing staff of ward 257A for their help in collecting urine samples.)

347.4 ONE-WAY GENERALIZATION OF CLONIDINE TO COCAINE IN RATS TRAINED ON A DISCRIMINATIVE STIMULUS PARADIGM. D. M. Wood*, M. W. Emmett-Oglesby, S. Yaden* and H. Lal. Department of Pharmacolgy, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107

Rats were trained to discriminate the stimulus properties of either cocaine or clonidine using a two-lever choice paradigm in which food reinforcement was delivered for responses on the correct lever: for the cocaine group, one lever was correct after cocaine (10 mg/kg) intra- peritoneal (i.p.) injection, and the other lever was correct after saline injection; for the clonidine group one lever was correct after clonidine (0.02 mg/kg) i.p. injection, and the other lever was correct after saline injection. After cocaine training, in a dose-dependent manner, cocaine (2.5-10 mg/kg) was generalized to the cocaine lever. After clonidine training, in a dose-dependent manner, clonidine (0.005- 0.02 mg/kg) was generalized to the clonidine lever. In addition, yohimbine, an alpha-2 antagonist blocked the clonidine generalization. In tests of cross-generalization, cocaine was not generalized to the clonidine stimulus; however, clonidine was generalized at low doses to the cocaine stimulus, and this generalization was blocked by yohimbine. The one-way generalization (clonidine to the cocaine stimulus, but not cocaine to the clonidine stimulus) suggests that clonidine has at least two discrete stimulus components: a major component that is not cocaine-like in character, and a minor component that can be detected by cocaine-trained subjects. In addition, the yohimbine blockade data suggest that both components of the clonidine stimulus are mediated via alpha-2 receptors.

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347.5 **EFFECTS OF 6-HYDROXYDOPAMINE LESIONS OF THE MEDIAL PREFRONTAL CORTEX ON INTRACRANIAL COCAINE SELF-ADMINISTRATION.** **N.E. GOEDERS and J.E. SMITH. Psychiatry Research Unit, Departments of Psychiatry and Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.**

The response-contingent delivery of cocaine to receptors in the medial prefrontal cortex initiates a reinforcing stimulus that is mediated in part through interactions with D_2-dopaminergic receptors (Goeders and Smith, Science, 221:773, 1983). This experiment was designed to directly investigate the involvement of the presynaptic dopaminergic innervations of this brain region in this behavior. Male Fischer F-344 rats were stereotaxically implanted unilaterally with guide cannulae into the medial prefrontal cortex. The animals were allowed to intracranially self-administer 100 nl microinfusions of cocaine HCl by lever-pressing during 8-hour sessions conducted every third day. Individual dose-response curves were generated, and the concentration resulting in maximum rates of responding (50 to 90 pmol) was determined and utilized for each rat. When stable baselines of drug-intake were observed, each animal received a unilateral 6-hydroxydopamine lesion (4 ug in 0.2 ul delivered over 6 min) at the site of cocaine self-administration. The lesion decreased drug-intake to vehicle levels and flattened the dose-response curve for each animal. Responding was reinitiated by substituting dopamine (300 pmol) for cocaine in the microinjection systems, and this effect was attenuated with equimolar concentrations of the D_2 receptor antagonist sulpiride. The substitution of serotonin (300 pmol) did not maintain responding at rates greater than vehicle. These data suggest that the dopaminergic innervations of the medial prefrontal cortex are critical for intracranial self-administration of cocaine. The drug probably interacts with presynaptic receptors on dopaminergic terminals to inhibit the reuptake of dopamine (Kennedy and Hanbauer, J. Neurochem., 41:172, 1983). The increased synaptic content of the neurotransmitter following cocaine administration may result in a potentiation of activity at postsynaptic D_2 receptors for the initiation of a reinforcing stimulus. **Supported in part by USPHS Grant MH-09222 (NEG) and USPHS Grant DA-01999 (JES).**

347.6 MESOCORTICAL DOPAMINE SYSTEM LESIONS DISRUPT COCAINE REINFORCED CONDITIONED PLACE PREFERENCE. W. Isaac,* J. Neiswander,* T. Landers,* R. Alcala,* M. Bardo and A. Nonneman (SPON: R. Yokel). Dept. of Psychology, Univ. of Kentucky, Lexington, KY 40506

The reinforcing efficacy of cocaine is thought to involve, at least in part, mesocortical dopaminergic neurons. Cocaine reinforcement is attenuated by dopamine antagonists such as pimozide, as well as by lesions of the ventral tegmental area. Rats will self-administer cocaine applied directly into the medial prefrontal cortex but not into nucleus accumbens or the ventral tegmental area (Goeders & Smith, Science, 221: 773-775, 1983). The present experiments assessed whether lesions of mesocortical target regions attenuate cocaine reinforcement.

Male Sprague-Dawley rats were anesthetized with chloral hydrate and the prefrontal cortical target of the mesocortical dopamine projection was removed by suction. At least one week postoperatively half the rats in each group received cocaine reinforced place preference conditioning. Every other day these animals received a subcutaneous injection of cocaine (2.5, 5.0, or 10.0 mg/Kg) and were placed in a small chamber with white walls, wire-mesh floor and pine bedding for 20 min. On alternate days these animals were injected with saline and placed in a small chamber with black walls, grid floor and cedar bedding for 20 min. The other half of the animals in each group received saline injections before being placed in either chamber. After 2, 6, 8, or 12 drug-placement pairings all animals were given free access to both chambers for 10 min. Time spent in each chamber was recorded.

The sham operated rats injected with cocaine spent significantly more time in the cocaine-associated environment than the sham operated rats injected with saline only. However, the time spent in the cocaine-associated environment did not differ from the time spent in the saline-associated environment for the animals with prefrontal lesions. This lack of place preference conditioning in the lesion subjects was evident at each dose of cocaine tested.

347.7 COCAINE CONDITIONING IN THE PLACE PREFERENCE PARADIGM: EFFECTS OF ENVIRONMENTAL REARING CONDITIONS. S. Schenk*, T. Hunt*,R. Malvechko* A. Robertson* and Z. Amit. Concordia University, Dept. of Psychology, Montreal, Quebec, H3G 1M8.

This experiment was designed to compare the effects of isolation and aggregation housing on the cocaine-induced conditioned place preference. Rats were obtained at weaning (21±2 days) and housed either in isolation or in groups of 4 per metal cage for a period of 6 weeks. The conditioned place preference paradigm consisted of 3 phases. In the first the rats were habituated to the experimental apparatus. On each of 4 days, they were permitted 15 min. access to a test box constructed of plywood with a Plexiglas top. The box was designed so that there were two distinct environments; the floor and walls on each side were unique. The floor was balanced such that if the rat moved to one end of the box, the floor tilted slightly,depressing a switch that activated a timing mechanism. The amount of time spent in each chamber was measured and the mean time on the last two days served as the pre-conditioning baseline score. During the second phase, the rats were injected, on each of 4 days, with cocaine HCl (0,0.31, 0.625, 1.25 or 2.5 mg/kg, i.p.) and confined to their initially nonpreferred side of the test chamber for 15 min. In the last phase (1 day) the rats were injected with vehicle solution and again permitted free access to the entire testing chamber for 15 min. The amount of time spent in the environment in which the rats experienced the consequences of the drug on conditioning days was compared to the amount of time spent in that environment during habituation.

Results indicated that group-housed animals were more sensitive to cocaine in this paradigm than were their isolation housed counterparts. Maximal place preference effects were obtained in the aggregated rats using the lowest dose of cocaine tested (0.31 mg/kg). In contrast, the isolation-housed rats failed to increase the percentage of time spent in the conditioned environment even at the highest dose tested (2.5 mg/kg). A 2-way ANOVA (dose X housing condition) performed on the difference scores (pre-conditioning - post-conditioning time) yielded a significant main effect of housing ($F(1,70)=5.64$, $p<.05$). Thus the rearing environment is an important factor that can influence the sensitivity of adult rats to the effects of cocaine in the place preference paradigm. We have previously shown this to also be true when heroin is used as the conditioning agent. This may indicate a generalized effect of early housing conditions on the sensitivity of mature rats to dependence-inducing drugs.

347.8 DISSOCIATION OF THE EFFECTS OF d-AMPHETAMINE ON THE DETECTION OF INTRACRANIAL STIMULATION FROM ITS PSYCHOMOTOR EFFECTS. J.E.G. Williams and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Detection thresholds for intracranial electrical stimulation were measured to determine the relative effects of d-amphetamine on the attention-discrimination capabilities in the rat. In addition to threshold determinations, intertrial responding and latency of response as a measure of the psychomotor effects of d-amphetamine, were recorded. The threshold level of stimulation for detection as employed in this procedure is by itself neither positively nor negatively reinforcing; however, this level of stimulation can be used as a discriminative stimulus in a simple instrumental task.

Male albino rats (CDF-Charles River Laboratories) were stereotaxically implanted with two bipolar stainless steel electrodes aimed at the mesencephalic reticular formation (MRF) and the medial forebrain bundle-lateral hypothalamic area (MFB-LH). Following surgery, the animals were trained to make an instrumental response to a non-contingent 0.5 sec MRF stimulation cue (S1). Responding to the cue within 5 sec was maintained by the delivery of a reinforcing contingent stimulus (S2) to the MFB-LH area. Absolute detection thresholds were determined by varying the current intensity of the brain stimulation cue (S1) according to a modification of the psychophysical method of constant stimuli. The contingent stimulus (S2) remained at a fixed highly rewarding intensity level. Preliminary results indicate that d-amphetamine (0.06-1.0 mg/kg) caused dose related monotonic increases in intertrial responding while the same dose range caused a slight but non-significant lowering of the detection threshold at doses of 0.06-0.25 mg/kg. In three of the four animals tested, a significant rise in the detection threshold was seen at doses of 0.5 or 1.0 mg/kg. Changes in attention-discrimination as measured by the detection threshold were clearly dissociated from increases in psychomotor activity and previously reported effects on reward thresholds. (Esposito, R., et al., Psychopharmacology, 69:187-191, 1980).

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347.9 $ACTH^{1-24}$ EFFECTS ON D-AMPHETAMINE SELF-ADMINISTRATION AND THE DYNAMICS OF BRAIN DOPAMINE IN RATS. A.P. Leccese and W.H. Lyness. Pharmacology Department, Texas Tech University Health Sciences Center, Lubbock, TX, 79430.

Experiments aimed at determining the neural basis of reward have previously focused on the role of neurotransmitters and have only recently begun to investigate the role of peptides. The present experiment investigated the effect of $ACTH^{1-24}$ on d-amphetamine self-administration in rats. Animals were trained daily (8 hour sessions) to press a lever which activated a system that administered 0.125 mg/kg of intravenous amphetamine. After achievement of a stable self-injection frequency, subjects were injected s.c. with 10 , 20 or 40 µg/80 µl $ACTH^{1-24}$ immediately prior to placement in the apparatus. The 20 µg and 40 µg doses of the peptide fragment induced a statistically significant attentuation of d-amphetamine self-injection which lasted for 2 days. Control rates of responding were achieved by 5 to 10 days after the peptide treatment. An experiment was conducted to evaluate possible neuromodulatory effects of the peptide fragment. Twenty four hr. after $ACTH^{1-24}$, HVA was elevated in the caudate. When both apomorphine and $ACTH^{1-24}$ were administered, the combination lowered HVA in the caudate to a greater degree than apomorphine alone. The peptide fragment, when combined with haloperidol, attenuated the haloperidol-induced increases of DOPAC and HVA in both the caudate and nucleus accumbens. It was tentatively concluded that the neuromodulatory action of $ACTH^{1-24}$ on dopaminergic neurons may result in an increase in the rewarding quality of d-amphetamine, thus rendering control level self-infusions superfluous. However, given the spectrum of physiologic activity of $ACTH^{1-24}$ in the body, exact mechanisms involved in the response suppression remain to be elucidated.

347.10 A COMPARISON OF THE REWARDING PROPERTIES OF "FREE" VERSUS "EARNED" AMPHETAMINE. M.M. La Cerra* and A. Ettenberg (SPON: G. Austin). Dept. Psychology, University of California, Santa Barbara, CA. 93106

Laboratory studies of drug reinforcement have, almost exclusively, involved investigations of the neurochemical substrates directly affected by the administration of the reinforcer. Such research has ignored the behavioral or operant response that generally precedes the delivery of the drug in these experiments. It is presumed that the response is important only as an index of the reinforcing quality of the drug. However, there is a growing literature that suggests that the response itself might contribute to the rewarding effects of positive reinforcers. In a variety of situations, animals prefer water, food or brain-stimulation rewards when they are self-administered (earned) as opposed to when they are provided noncontingently (free). In the present study we investigated whether contingent drug reinforcement might also be more rewarding than the same reinforcer delivered noncontingently.

Ten male albino rats served as subjects. On a given day half the rats ran down a straight alley to a distinctive goal box where each received a 1.0 mg/kg i.p. injection of d-amphetamine. For some animals the goal box was black in color, had a smooth Plexiglas floor and an acetic acid odor. For others the goal box was white, had a soft floor of animal bedding and contained no acetic acid odor. On alternate days each animal was injected with the same dose of amphetamine and placed into the opposite colored chamber to the one that they had run to on the previous day. The goal boxes and test procedures were counterbalanced and in each case the rats remained in the test environment for 30 min post-injection.

Every four days a 10 min place preference test was conducted by placing an animal into a rectangular box that offered him a choice between the two environments that he had experienced during training. Prior to the experiment, a preliminary preference test confirmed that animals did not have inherent preferences for one or the other side of the test box. However, once treatment had commenced, there was a highly reliable shift in place preference for the environment in which rats had received contingent amphetamine over the environment in which they had received noncontingent amphetamine. The size of these preferences remained stable over 16 days of drug treatment. These data suggest that response factors can contribute to the rewarding experience produced by positive reinforcing psychoactive drugs.

347.11 HALLUCINOGENIC AMPHETAMINE SELECTIVELY DESTROYS BRAIN SEROTONIN NERVE TERMINALS. G.A. Ricaurte*, L.S. Seiden and C.R. Schuster (SPON: R.J. Dinerstein). Dept. Pharmacol. and Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

3,4-Methylenedioxyamphetamine (MDA) is a synthetic amphetamine derivative which possesses hallucinogenic as well as psychomotor stimulant activity. It is a popular illicit drug within the drug subculture. The purpose of this study was to assess the neurotoxic effects of MDA on the dopamine (DA), serotonin (5HT) and norepinephrine (NE) systems in the rodent brain. Male albino rats were administered various subcutaneous doses of MDA every 12 hours for 4 days and killed 2 weeks later. Regional brain monoamine level determinations at this time showed that MDA, in doses as low as 5 mg/kg/day x 4,produced a selective long-lasting depletion of 5HT in various brain regions (hippocampus, striatum) without affecting the level of DA or NE. This persistent 5HT depletion was accompanied by a loss of 5HT uptake sites and a decrement in 5-hydroxyindoleacetic acid concentration. Furthermore, these 5HT neurochemical deficits were correlated with morphological evidence of 5HT terminal degeneration. Finally, it was found that repeated doses of MDA were not necessary to induce 5HT neurotoxicity as a single 10 mg/kg dose of MDA was sufficient to produce comparable 5HT nerve terminal degeneration. These results demonstrate that MDA, the prototype of the group of ring-substituted amphetamines with hallucinogenic activity, destroys rat brain 5HT nerve terminals and raises the question of whether MDA use by man results in brain 5HT nerve terminal destruction. G.A.R. supported by USPHS 5 GM-07190; L.S.S. and C.R.S. by DA-00250 and DA-00085.

347.12 INFLUENCE OF HIERARCHIAL RANK ON BEHAVIORAL RESPONSE TO CHRONIC d-AMPHETAMINE TREATMENT IN SELECTED MEMBERS OF STUMPTAIL MACAQUE SOCIAL COLONIES. R.F. Schlemmer, Jr. & J.M.Davis. Illinois State Psychiatric Institute, Chicago, IL 60612

Frequent, repeated administration of amphetamine often leads to the development of amphetamine psychosis, a syndrome similar to paranoid schizophrenia. Subsequently, behavior induced by chronic administration of amphetamine to animals is commonly used as animal model of schizophrenia. Amphetamine-induced behavior in selected members of primate social colonies has been a particularly relevant model due to the similarity of several behavioral changes to human schizophrenic behavior. Like human amphetamine psychosis there appears to be some variation in response between individual subjects. One possible explanation comes from recent reports from several laboratories which suggest that hierarchial rank within a social colony may be an important determinant of behavioral response to d-amphetamine (AMPHET). This report documents rank-dependent changes induced by chronic AMPHET treatment in 15 female members of seven adult Stumptail macaque (Macaca arctoides) social colonies. Each experiment began with observation of undrugged behavior. This was followed by chronic administration of d-amphetamine. 1.6 mg (base)/kg in time-release form, nasogastrically every 12 hrs for 12 consecutive days. Two members from each colony of 4-5 animals received drug treatment. A "blind" observer quantitated & recorded the behavior of each monkey in the colony for 60 min each day. Hierarchial rank was determined daily by the observer using recognized determinants of rank in this species. Predominant behavioral changes seen with chronic AMPHET Tx included the induction of stereotyped behavior, increased checking, social withdrawal, increased submissive behavior, and increased scratching. Rank-dependent changes noted were as follows. Dominant females: 1) exhibited social forms of stereotypy whereas lower ranking females did not, 2) had significant increases in submissive gestures whereas lowest rank-females did not, 3) developed stereotyped threatening behavior, and 4) had significant increases locomotion. Lower ranking subordinant females: 1) became spatially isolated early in AMPHET Tx & 2) had significantly larger increases in self-grooming. AMPHET-induced increases in general activity, overall stereotypy, checking, and scratching did not appear to be rank-dependent. These results demonstrate that hierarchial rank is an important variable in several prominent behavioral changes induced in Stumptail macaque monkeys by chronic d-amphetamine treatment.

347.13 COCAINE ANTAGONISM OF γ-AMINOBUTYRIC ACID ACTION ON CULTURED HIPPOCAMPAL NEURONS. S.A. Cohen. Departments of Anesthesiology and Pathology, Mount Sinai School of Medicine, New York, New York 10029.

Cocaine has been used clinically for years, but its illicit abuse for central nervous system (CNS) stimulation has increased dramatically recently. Despite extensive investigation the cellular mechanisms of cocaine's CNS stimulatory effects remain unknown. The present research describes cocaine's antagonism of the action of the putative inhibitory CNS neurotransmitter γ-aminobutyric acid (GABA).

Primary dissociated cell (PDC) cultures of hippocampal neurons (HN) prepared from 14-16 day old mouse fetuses were studied electrophysiologically after 1-3 weeks in culture. HN were penetrated for intracellular recording with glass micropipettes. Microiontophoretic pipettes identical to those used for voltage recording were filled with GABA (0.5M, pH 2.9). Cocaine (100 nM) was added to a carrier solution identical to the RS and delivered from small bore pipettes which were progressively brought to within 5 um of an impaled HN.

The average resting membrane potential (RMP) of HN was -51.82$\pm$9.81 mV (mean $\pm$ S.D.). All HN tested were sensitive to iontophoretically applied GABA. GABA responses from a single cell were stable over time. Cocaine applied by passive diffusion diminished the GABA response. The GABA response was depressed as a function of cocaine concentration. As the cocaine pipette was advanced towards the HN soma, the GABA response progressively declined until it was completely abolished. This effect was long lasting; when the cocaine pipette was withdrawn, the cell remained unresponsive to the same dose of GABA for about three minutes. The response slowly increased in amplitude with time. In some cases cocaine caused an increase in RMP after the GABA response was attenuated.

Since GABA is thought to be an inhibitory neurotransmitter in HN, the results strongly support the hypothesis that cocaine acts as a modulator of neurotransmission in the hippocampus. Although a variety of compounds have been shown to alter cholinergic synaptic function, this is the first demonstration in which a local anesthetic modulated GABA mediated responses. Thus, the action of cocaine is qualitatively similar to a variety of other agents which produce neuronal excitability. This may account not only for cocaine's CNS euphorigenic action but also for its ability to produce seizures.

347.14 ALTERATIONS IN GLUCOSE UPTAKE IN BRAIN IN DIAZEPAM-DEPENDENT AND DIAZEPAM-WITHDRAWING RATS. Cheryl A. Marietta*, Michael J. Eckardt, Kerry L. Zbicz*, and Forrest F. Weight. Lab. of Preclinical Studies and Lab. of Clinical Studies, Nat'l. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD 20852

Diazepam can produce physical dependence as revealed by withdrawal symptoms characterized by hyperactivity (Rastogi, et. al., J Psychiat Res 13: 65, 1976). We investigated the effect of diazepam dependence and withdrawal on cerebral glucose metabolism using 2-deoxy-D[^{14}C] glucose (2-DG) as a metabolic tracer. Female Sprague-Dawley rats were rendered physically dependent by oral administration of a diazepam suspension twice a day, seven days a week, on a rapidly increasing dosage schedule from 20 mg/kg/day to 100 mg/kg/day, then maintained on 100 mg/kg/day for 5 weeks. Physical dependence was indicated by the appearance of a withdrawal syndrome characterized by tremors, hyperactivity and weight loss. The symptoms started approximately 36 to 40 hours after the last dose and peaked 12 to 20 hours after the onset of symptoms. Animals were prepared for the determination of local brain glucose uptake using the 2-DG method of Sokoloff et. al. (J. Neurochem, 28: 897, 1977) either 2 hours after the last dose of diazepam (dependent) or when a maximal withdrawal response was noted (withdrawing). The autoradiographs from dependent rats appeared significantly lighter than the controls, indicating a decrease in 2-DG uptake. The decrease in uptake appeared to be a generalized depression of uptake as no specific localized changes in uptake patterns were observed when comparing the dependent and control rats. Although the autoradiographs of withdrawing rats did not appear to show a generalized change in 2-DG uptake, localized changes were observed: columnar areas of increased uptake were apparent in the frontal sensorimotor cortex, ovoid areas of increased uptake could be seen in the cerebellum, and the lateral geniculate of the withdrawing rat showed increased uptake in the dorsal portion. The localized areas of increased uptake seen in autoradiographs from withdrawing rats were not apparent in either the control or dependent rats. Our results are consistent with a decrease in CNS activity in dependent rats. During both ethanol and phenobarbital withdrawal there is a generalized increase in 2-DG uptake (Campbell, et. al., Brain Res, 237 : 571, 1982; Marietta, et. al., Neurosci Abs 9, 1238, 1983). Our results indicate that there does not appear to be a generalized increase in uptake of 2-DG during withdrawal from diazepam.

347.15 LABELLING OF CANNABINOID BINDING SITES IN BRAIN WITH A ^{3}H-QUATERNARY AMMONIUM ANALOGUE OF DELTA-8 THC. J.S. Nye, H.H. Seltzman*, C.G. Pitt* and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins University, Sch. of Medicine, Baltimore, MD 21205; *Research Triangle Institute, Research Triangle Park, NC 27709.

We have studied the in vitro binding of ^{3}H-5'-trimethyl ammonium delta-8 THC (^{3}H-TMA) to rat neuronal membranes. TMA is a positively charged analogue of THC modified in the aliphatic side-chain, a portion of the molecule not important for its psychoactivity. Unlabelled TMA inhibits field-stimulated contractions of the guinea pig ileum at low concentrations (IC_{50} = 0.9 μM) and in the same presynaptic manner at delta-9 THC.

^{3}H-TMA binds saturably to brain membrane preparations. TMA binding is reversible, sensitive to proteases, boiling and shows a pH maximum around 7. Additionally, treatment of membranes with numerous detergents, including Triton X-100 and CHAPS, will solubilize the sites without altering their pharmacological properties.

Delta-9 THC competitively inhibits ^{3}H-TMA binding potently (K_i = 10 nM) and stereoselectively. While many cannabinoids also inhibit TMA binding, no other drug or unrelated compound tested so far has comparable potency. For many cannabinoids potency in behavioral and physiological tests parallels their potency at the ^{3}H-TMA binding site. However, several behaviorally inactive cannabinoids such as cannabinol and cannabidiol are active at the binding site. These drugs may behave as antagonists to delta-9 THC as suggested by physiological and behavioral studies (Krantz et al., Am. J. Pharm., 1971:149-152, 1971; Karniol and Carlini, Psychopharmacologia, 33:53-70, 1973; Karniol et al., Eur. J. Pharmacol., 28:172-177, 1974).

347.16 GTP REVERSES THE EFFECTS OF Δ^9-TETRAHYDROCANNABINOL ON THE BINDING OF ANTAGONISTS TO THE β-ADRENERGIC RECEPTOR. Cecilia J. Hillard and Alan S. Bloom. Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

We have demonstrated previously that Δ^9-tetrahydrocannabinol (THC) increases the binding of antagonists to the β-adrenergic receptor of mouse cerebral cortical membranes (Hillard, C.J. and Bloom, A.S., Soc. Neurosci Abst. 7: 448, 1981). In the presence of 10 μM THC, ^{3}H-dihydroalprenolol (^{3}H-DHA) binding is described by a right-shifted, curvilinear Scatchard plot. ^{3}H-DHA binding in the presence of THC was best fit to two binding sites, a high affinity site with a K_D=0.06 nM, accounting for 10% of the binding and a low affinity site with a K_D=6.7 nM, accounting for 90% of the binding. In the presence of vehicle alone, the K_D was 2.61 nM.

The binding of dl-propranolol (PROP) was affected by THC in an analogous manner. PROP displacement curve in the presence of 10 μM THC had an IC_{50} of 16.2 nM, compared to 32.0 nM in the presence of vehicle. The Hill coefficient was 0.58 in the presence of THC, which is indicative of multiple sites. Nonlinear curve fitting techniques confirm a two site model with a high affinity site (K_I=0.08 nM) accounting for 16% of the total sites and a low affinity site (K_I=5.96 nM) accounting for 84% of the total sites. PROP binding in the presence of vehicle alone was best described by a one site model (K_I=6.55 nM).

One explanation for the appearance of this high affinity site is THC stabilization of the receptor Ns protein complex. In this situation, antagonists would bind to both the low affinity, uncoupled receptor and the high affinity "precoupled" receptor. If this hypothesis is correct, the high affinity site seen in the presence of THC ought to disappear in the presence of GTP.

GTP reverses the effects of THC on both ^{3}H-DHA binding and on PROP binding. In the presence of GTP and THC, ^{3}H-DHA binding is to a single site, as demonstrated by a linear Scatchard plot (K_D=2.93 nM). PROP displacement curve in the presence of THC and GTP has an IC_{50} of 31.3 nM and a Hill coefficient of 0.85. Nonlinear curve fitting of the data indicates a single site with a K_I=4.68 nM.

These results indicate that the effect of THC on antagonist binding to the β-adrenergic receptor is consistant with a hypothesis of THC stabilization of a high affinity, receptor Ns precoupled state and that this site is GTP sensitive, similar to the ternary complex binding site. (Supported by USPHS Grant DA-00124).

347.17 (+)-CATHINONE AFFECTS DOPAMINE AND 5-HYDROXYTRYPTAMINE NEURONS IN RAT BRAIN. J.A. Nielsen. Pharmacology Program, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

(+)-Cathinone is an active ingredient in the leaves of the khat shrub. (+)-Cathinone affects behavior (Schechter et al., Pharmacol. Biochem. Behav. 20:181, 1984), neurochemistry and electrophysiology (Mereu et al., Life Sci. 32:1383, 1983) in a manner similar to the stimulants amphetamine, cocaine and methylphenidate. The present study extended these studies by evaluating the effects of (+)-cathinone on dopamine (DA), 5-hydroxytryptamine (5-HT), and norepinephrine (NE) neurons in several rat brain regions in vivo.

The in vivo rate of DA, 5-HT and NE synthesis was determined in the nuclei caudatus putamen (CP), accumbens (NA), amygdaloideus centralis (AC), septi lateralis (SL), preopticus pars suprachiasmatica (PSCN), and dorsomedialis (hypothalami) (DMN) of male rats (175-225g) by measuring the concentration of dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP) after the administration of NSD 1015 (100 mg/kg, i.p.) an inhibitor of aromatic L-amino acid decarboxylase (Nielsen et al., Life Sci. 33:1899, 1983). Concentrations of DA, 5-HT, NE, and their major metabolites dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA), and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), respectively, were analyzed by high pressure liquid chromatography coupled to an electrochemical detector.

(+)-Cathinone decreased DOPAC in a time- and dose-related manner in the CP, NA, AC, and SL with the peak effect occuring 30-60 minutes after a dose of 6 mg/kg (i.p.). (+)-Cathinone had no effect on DOPAC in the PSCN or DMN. The drug also decreased the accumulation of DOPA in the CP, NA, AC, and SL, but in the PSCN and DMN their was no effect. (+)-Cathinone also decreased 5-HIAA and 5-HTP in the CP, NA, AC, and SL, but was without effect in the PSCN and DMN. (+)-Cathinone was less potent in affecting the 5-HT neurons as compared to the DA neurons. Finally, (+)-cathinone did not alter the amount of MHPG in any region studied.

These results suggest that (+)-cathinone: 1) affects DA neurons in forebrain, but not hypothalmic regions; 2) alters forebrain, but not hypothalamic 5-HT neurons less potently than DA neurons; and 3) does not affect NE neurons. (Supported by Northeastern Ohio Universities College of Medicine BMS grant 2380 and NIDA grant 03591-01).

347.18 EFFECT OF THC ON MEDIAL SEPTAL FACILITATION OF THE FACIA DENTATA SPIKE RESPONSE TO PERFORANT PATH STIMULATION. N.J. Pontzer,* and D.M. Wilkison* (SPON: M.J. Hosko). Dept. of Pharm. and Tox., Medical College of Wisconsin, Milwaukee, WI 53226.

Although the precise mechanisms by which tetrahydrocannabinol (THC) produces its unique behavioral effects are not known, there is evidence suggesting an involvement of central cholinergic systems. THC has been shown to decrease acetylcholine release, turnover and uptake in the cortex and hippocampus, possibly by a pre-synaptic action. Hippocampal theta rhythym, which may be in part mediated by cholinergic septo-hippocampal projections is sensitive to disruption by THC. Both the hippocampus and central cholinergic systems have been implicated in learning and memory. THC and anti-cholinergic agents produce similar deficits in short term memory. It was thus of interest to study the effect of THC on cholinergic modulation of hippocampal function. Other work in this area has shown a direct augmentation of the monosynaptic response at CA1 and dentate in vitro and at CA1 in vivo by THC.

Male Sprague-Dawley rats (250-400g) were anesthetized with urethane (1.5-2.0 g/kg), mounted in a stereotaxic frame and the skull opened over the perforant path (PP), facia dentata (D) and medial septal (MS). Concentric bipolar stimulating electrodes were placed into PP and MS and an insulated tungsten wire recording electrode into D. Various length (15-100 mSec) and frequency (100-250 Hz) trains of conditioning stimuli were delivered to MS immediately previous to PP stimulation. Three levels of PP and MS stimulation were used. Two control periods and three periods 15 min post cumulative THC i.v. (1, 3 and 10 mg/kg), were measured. Eight successive evoked dentate responses were averaged and the population spike measured by computer after high pass digital filtering (160 Hz) to eliminate the low frequency EPSP. Stimuli were delivered at 0.2 Hz.

Low doses of THC appeared to decrease or abolish the population spike augmentation produced by prior MS stimulation. In some animals, THC directly augmented the population spike in the absence of MS stimulation. A variable low amplitude direct response to MS stimluation at the dentate was also abolished in a few animals in which this was measured. An action of THC to directly augment monosynaptic response in the hippocampus while decreasing the facilitation produced by MS stimulation could account for some of its behavioral actions.

347.19 THE EFFECT OF THE GABA AGONIST MUSCIMOL ON 5-MeODMT-INDUCED BEHAVIORAL CHANGES ON SELECTED MEMBERS OF A PRIMATE SOCIAL COLONY. C. Nawara, R.F. Schlemmer, Jr., W.J. Heinze*, J.M. Davis. Ill. State Psychiatric Institute, Chicago, IL 60612.

5-Methoxy N,N-dimethyltryptamine (5-MeODMT) is a hallucinogen which induces several behavioral changes in primates which have been proposed as a model for the study of psychotic processes. We have previously reported that 5-MeODMT-induced emergent behaviors can be significantly influenced by pharmacologic alteration of several neurotransmitter systems, particularly by serotonin and dopamine antagonists. The present study examined the influence of the GABA agonist muscimol (MUS) on 5-MeODMT-induced primate behavior. Interestingly, intoxication with some GABA agonists such as MUS also results in hallucinations, yet an interaction between these agents and recognized hallucinogens has not been tested. In the study, MUS and 5-MeODMT were administered alone and in combination to 4 selected members of an adult Stumptail macaque social colony. The experiment began with observation of non-drugged behavior (BASE) followed by drug treatment. Each monkey received 5-MeODMT (0.25 mg/kg) and MUS (1.0 mg/kg) alone and in combination i.m. in a cross-over design. Two monkeys received drug treatment per day while the other two animals received saline. A 60 min. behavioral observation was conducted by a "blind" observer beginning 5 min after 5-MeODMT or saline injection (20 minutes after MUS administration). Given alone, 5-MeODMT induced two emergent behaviors, limb jerks and body shakes. This hallucinogen also significantly increased checking and food forage and decreased social grooming. MUS significantly decreased social grooming and activity and induced emesis. However MUS did not significantly alter other behaviors. Given in combination MUS failed to alter most 5-MeODMT-induced behavior. Limb jerks and body shakes were still significantly increased from BASE levels but were not significantly different from 5-MeODMT alone. Checking scores also remained significantly elevated from BASE, while social grooming was eliminated by the combination. These results suggest that GABA systems do not appear to play a major role in the modulation of primate behavior induced by the hallucinogen 5-MeODMT. Further, these two hallucinogenic agents do not appear to exert their behavioral effects through a common pharmacologic mechanism in primates.

347.20 EVIDENCE FOR SPONTANEOUS, BUT NOT PRECIPITATED, WITHDRAWAL FROM NICOTINE IN RATS. M. W. Emmett-Oglesby, C. M. Harris, N. G. Robinson* and H. Lal. Department of Pharmacology, Texas College of Osteopathic Medicine, Ft Worth, TX 76107.

Previous work from this laboratory has demonstrated that withdrawal from diazepam or morphine is associated with the occurrence of an interoceptive stimulus that has a property similar to that of the anxiogenic drug pentylenetetrazol (PTZ). This experiment tested whether withdrawal from chronic nicotine would also produce a similar interoceptive stimulus. Rats were trained to press a lever using food as a reinforcer. They were then trained to discriminate an injection of PTZ, 20 mg/kg, from an injection of saline, using a two-lever choice procedure in which the correct lever was determined by injection condition and was reinforced under a fixed-ratio 10 schedule. Prior to chronic injection conditions, rats chose the PTZ lever following PTZ in a dose-dependent manner, and nicotine was partially generalized to the PTZ stimulus with 38% of subjects selecting the PTZ lever following 0.64 mg/kg nicotine. Diazepam, 5 mg/kg, blocked the stimulus produced by PTZ and the PTZ-like stimulus produced by nicotine. Mecamylamine, 5.0 mg/kg also blocked the PTZ-like stimulus of nicotine. Chronic nicotine was given for 22 days. Rats received nicotine by subcutaneous injection at doses of 0.64 mg/kg tid on day 1 and 1.25 mg/kg tid on succeeding days. During chronic administration, mecamylamine (1.25, 2.5 or 5.0 mg/kg) did not increase the selection of the PTZ lever. In contrast, a PTZ-like stimulus was present in approximately 35% of subjects 8-hours after nicotine injections. At 24 and 48 hours after termination of chronic nicotine, 50% of subjects selected the PTZ lever when injected with saline, and this stimulus was detected to a lesser degree over the next 4 weeks. During this time, the withdrawal stimulus was shown to be additive with the stimulus produced by PTZ, and was blocked by either diazepam or triazolam, confirming that the partial generalization was an accurate detection of a PTZ-like stimulus occurring during spontaneous withdrawal. Thus, these results confirm that the discrimination of PTZ has broad utility for investigating subjective aspects of drug withdrawal in animals.

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REGULATION OF PITUITARY FUNCTION V

348.1 INFUSION OF VASOACTIVE INTESTINAL PEPTIDE INTO THE THIRD VENTRICLE INHIBITS PULSATILE LH SECRETION IN OVARIECTOMIZED RATS. M.J. Alexander*, D.K. Clifton*, and R.A. Steiner, Departments of Physiology and Biophysics, and Ob-Gyn, University of Washington, Seattle, WA 98195

A number of recent studies provide evidence implicating vasoactive intestinal peptide (VIP) in synaptic function, either as a transmitter or neuromodulator. VIP-like immunoreactivity has also been demonstrated in brain regions implicated in the regulation of gonadotropin-releasing hormone (GnRH) secretion. In addition, VIP has been reported to influence luteinizing hormone (LH) secretion. Therefore, we tested the hypothesis that VIP acts at the brain to alter pulsatile GnRH release, as reflected in pulsatile LH secretion.

We implanted chronic third-ventricular cannulae in long-term ovariectomized (OVX) rats at least 7 days prior to testing. Discrete 300µl blood samples were taken from conscious, unrestrained animals at 5 min intervals for a total of 4 hr. Each sample was replaced with an equal volume of a blood replacement mixture. Immediately following a control bleeding period of 2 hr, saline or VIP (in saline at a dose of 2nmole/15µl/hr) was infused into the third ventricle (icv) for 2 hr. VIP infusion (n=6) resulted in a 78% decrease in LH pulse frequency (from 5.8 $\pm$ 0.7 to 1.2 $\pm$ 0.4 pulses/2 hr; $p < 0.001$), a 58% decrease in mean plasma LH levels (from 8.6 $\pm$ 1.1 to 3.5 $\pm$ 0.6 ng RP2/ml; $p < 0.001$), and no significant change in LH pulse amplitude when compared with preinfusion values. Saline infusion (n=5) yielded no statistically significant changes in any of these parameters. To assess whether VIP had a direct pituitary effect, we challenged VIP-treated animals with GnRH (10ng/ml, iv) just before the end of the icv infusion. We observed a pronounced increase in plasma LH levels (from 3.0 $\pm$ 1.1 to 14.5 $\pm$ 2.3 ng/ml) in response to this challenge.

These results demonstrate that icv infusion of VIP in OVX rats profoundly inhibits LH pulses as it reduces mean plasma LH levels. The potency of the VIP dose used in this study was at least one order of magnitude greater than that exhibited by norepinephrine in a previously reported study of similar design. That exogenous GnRH elicited an LH response in VIP-treated animals argues against a direct effect of VIP on the pituitary gonadotropes in this experiment. Conclusion: VIP may act as an important inhibitory neurotransmitter for the regulation of GnRH secretion.

348.2 APOMORPHINE-INDUCED INHIBITION OF PULSATILE LH RELEASE IN MSG-TREATED RATS. P. A. Rose* and R. F. Weick*, Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1. (SPON: R. R. Shivers)

The role of dopamine in the regulation of luteinizing hormone (LH) secretion has been widely debated. Administration of the dopamine receptor agonist apomorphine results in a transient but marked inhibition of pulsatile LH discharges (Drouva, S.V. and Gallo, R.V., Endocrinology 99:651, 1976). Neonatal male rats were treated with monosodium-L-glutamate, which has been shown to damage the arcuate nuclei and decrease dopamine stores in the mediobasal hypothalamus; control rats were treated with equiosmolar injections of saline. The rats were castrated as adults, chronic venous catheters were installed, and blood samples were taken every 5 minutes for a 3 hour period. After one hour of blood sampling, the animals were given an i.p. injection of either a small dose of apomorphine (0.8 mg/kg) or an equivalent volume of saline. LH was measured in the blood samples by RIA. Saline injection did not affect pulsatile LH patterns or LH concentrations. Although apomorphine had no effect on LH patterns in the rats which had received neonatal saline treatment, there was a striking inhibition of circulating LH levels in the MSG treated rats, which lasted 83.4 ± 8.3 min (mean ± S.E.). These results suggest that the depletion of dopamine which followed the MSG treatment resulted in a supersensitivity to the dopamine agonist. Furthermore, they add to the evidence implicating tuberoinfundibular dopamine as an inhibitory regulator of LH secretion.
(Supported by MRC of Canada.)

348.3 NALOXONE DISINHIBITS PULSATILE LH SECRETION DURING ESTRUS OF THE RAT ESTROUS CYCLE BUT DOES NOT STIMULATE LH RELEASE DURING LACTATION. S.R. Fox, M.T. Hoefer* and M.S. Smith*. Dept. Physiology, Univ. Pittsburgh, Pittsburgh, PA 15261.

These studies examined the involvement of endogenous opioids in the inhibition of basal pulsatile LH secretion during estrus of the estrous cycle and during lactation. The pattern of LH secretion was described by collecting blood samples every 10 min for 4h from chronic venous catheters and analyzing the plasma for LH concentrations. The lack of pulsatile LH secretion during estrus (mean LH, 7.5 ng/ml) was not due to a suppression of pituitary responsiveness to GnRH since the pulsatile administration of the opioid antagonist naloxone (0.5 ng/pulse every 50 min) to estrous animals induced an LH pulse in response to each pulse of naloxone (LH peak amplitude = 36 + 7 ng/ml). To determine whether continuous naloxone infusions could unmask endogenous pulsatile GnRH secretion on estrus, naloxone was administered via a second chronic venous catheter (loading dose, 1 mg; infusate, 0.7 mg/hr). In response to continuously elevated levels of naloxone, 12 of 17 estrous animals exhibited distinct pulses of LH secretion. The LH pulse amplitude of 65 + 6 ng/ml was somewhat greater than the average LH pulse amplitude observed during the rat estrous cycle. However, the LH interpulse interval of 56 + 4 min in the naloxone-infused estrous animals was similar to the 50 + 4 min interval previously described by us for the endogenous LH pulses occurring during the other days of the rat estrous cycle. The effect of naloxone administration during lactation was studied on day 10 postpartum in females nursing 2 pups. LH release before and after naloxone treatment in females nursing 2 pups was nonpulsatile (mean = 6 ng/ml). The lack of response to naloxone was not due to an inability of the pituitary to respond to GnRH. Administration of pulsatile GnRH (0.4 ng/pulse every 50 min) resulted in LH pulses of 21 + 3 ng/ml. These results demonstrate that despite the similarities in the pattern of LH release in both lactating and estrous rats, different mechanisms underlie the inhibition of LH pulses found during these 2 physiological states. During lactation, pulsatile LH secretion is inhibited by a naloxone insensitive mechanism. In contrast, during estrus, the inhibition of pulsatile LH release may be due entirely to opioid mechanisms acting to inhibit the synaptic release of GnRH but not to modulate GnRH pulse frequency.

348.4 PRENATAL DEVELOPMENT OF LHRH NEURONS IN THE HYPOTHALAMUS OF THE GALAGO CRASSICAUDATUS AND THE RHESUS MACAQUE. O.K. Rønnekleiv, D.L. Hess*, J.A. Resko* and W.E. Ellinwood* (SPON: W.R. Woodward). Or. Health Sciences Univ., Portland, Or. 97201, and Or. Regional Primate Res. Ctr., Beaverton, Or. 97006.

Previously we have shown a marked sex difference in serum gonadotropin concentrations in fetal rhesus macaques, and have demonstrated that the gonadal-hypothalamo-pituitary negative feedback system is fully operative as early as midgestation. Little is known about the ontogeny of hypothalamic peptides in either this species or in prosimians also reported here. In this study we have used immunohistochemical methods to determine the time of appearance of LHRH neurons in the hypothalamus, and also the occurrence of cells in the pituitary containing LH and FSH. The galago fetuses ranged in age from about 36 to 128 days (n=25) and the rhesus fetuses from 47-140 days (n=6). In each experiment the brain was immediately removed from the skull, divided into 1 mm thick coronal or sagittal blocks and immersed in the 4% paraformaldehyde for 3 hrs. The pituitaries were fixed for 2 hrs. Following fixation the tissue blocks were rinsed overnight in phosphate buffer containing 10% sucrose. Thereafter, the brain and pituitary tissues were sectioned on a cryostat, mounted on gelatinized slides and processed by the PAP method for LHRH, LH and FSH. The LHRH antiserum (EL-14) was used at a dilution of 1:2000, hLH and hFSH (β-subunits) at 1:800. Although few in number, distinctive LHRH neurons were present in the hypothalamus of 40-day-old galago fetuses. Furthermore, extensive LHRH neuronal stain was found in the hypothalamus of a 47-day-old rhesus female. The cell bodies were scattered dorsal, rostral, and caudal to the optic chiasm. In both galago and rhesus fetuses there was a gradual increase in the number of LHRH neurons observed in the hypothalamus with increasing age. By 120 days of gestation very high concentration of LHRH neurons were found in the preoptic area and a large number of cell bodies were also located in the arcuate and the lateral tuberal region of the MBH.

Examination of pituitaries from 40-50 day galago and rhesus fetuses showed no gonadotropin staining, although light staining was observed by 60 days and this increased in intensity by 80 days. These data support the hypothesis that hypothalamic regulating factors appear before or concurrent with the synthesis of pituitary hormones.
Supported by NIH grants NS18848 and HD16793.

348.5 RESPONSE OF IDENTIFIED MEDIAL BASAL HYPOTHALAMIC NEURONS TO NOREPINEPHRINE AND ESTROGEN: AN IN VITRO ELECTROPHYSIOLOGICAL STUDY T.P. Condon*, O.K. Rønnekleiv and M.J. Kelly Dept. of Physiology, Oregon Health Sci. U., Portland, OR

Both noradenergic input and estrogen feedback are critical components in the control of gonadotropin secretion in the mammal. Presently, we have utilized the in vitro hypothalamic slice preparation from the female guinea pig to study the effects of norepinephrine (NE) and 17β-estradiol (E_2) on the electrical excitability of LHRH neurons while simultaneously measuring the peptidergic output from the slices. Intact cycling and ovariectomized E_2-primed guinea pigs were decapitated and 400-600 μm sagittal hypothalamic slices were prepared (Kelly et al., Brain Res. Bull. 12(2): 1984). After 2 hr equilibration in the chamber both the recording and collection of the effluent medium (15 min samples of 800-1200 μl) were initiated. Procion yellow (PY)-filled and KCL/KCitrate-filled electrodes were utilized for intracellular recordings from arcuate (ARC) and cell-poor zone (CPZ) neurons. NE (10^{-7} to 10^{-4}M) and E_2 (10^{-8}M) were applied via the medium. Most of the cells were injected with PY and the slices were fixed and processed for LHRH immunocytochemistry. Twenty ARC-CPZ neurons were recorded (RMP's of -40 to -70mV) and tested with NE. Two of the cells were driven orthodromically by median eminence stimulation. A number of cells exhibited electrophysiological characteristics of peptidergic neurons (plateau potentials with bursts; afterhyperpolarizations). Fourteen of the tested neurons exhibited a dose-dependent inhibition of spontaneous activity. Four NE-inhibited cells were tested with E_2 and were inhibited (N=2) or uneffected (N=2). Four other ARC-CPZ neurons were excited by NE and two of these were found to be inhibited by E_2. The remaining two ARC-CPZ neurons tested with NE were not effected. The majority of tested neurons were PY identified and one of these cells (inhibited by both NE and E_2) was immunoreactive for LHRH. Furthermore, the release of the peptide in vitro appears to be similar to in vivo LHRH release and dependent on the steroid milieu. These studies demonstrate that the majority of ARC-CPZ neurons are inhibited by NE and that NE and E_2 actions are exerted on neurons within the medial basal hypothalamus. Experiments are in progress to ascertain the exact nature of the inhibition and how it affects the neurosecretory release of LHRH.
(Supported by PHS Grants NS 18989 and Fellowship HD 06332)

348.6 COITAL STIMULI CONTROLLING LUTEINIZING HORMONE SECRETION IN THE FEMALE FERRET. R.S. Carroll*, M.S. Erskine, L.A. Lundell*, P.C. Doherty and M.J. Baum. Dept. of Nutrition & Food Science, M.I.T., Cambridge, MA 02139.

A series of experiments focussed on the behavioral stimuli controlling pituitary luteinizing hormone (LH) secretion and ovulation in the female ferret. An initial experiment investigated which coital stimuli from the male are required to induce ovulation. It was found that female ferrets ovulated (verified by counting corpora lutea) only if the male achieved an intromission; neck gripping, mounting and pelvic thrusting behavior without an intromission by the male failed to induce ovulation. Subsequent experiments investigated the effect of gonadotropin releasing hormone (GnRH) on LH secretion, and the timing and magnitude of the coitus-induced LH surge associated with ovulation. Blood was withdrawn from females via intrajugular cannulae after infusion of GnRH as well as in various mating situations. LH was measured by radioimmunoassay. It was found that infusion of GnRH (4μg) caused a three-fold increase in plasma LH, which peaked in about 15 minutes and returned to baseline after an additional 45 minutes. After mating significant peaks in plasma LH occurred only when an intromission was achieved by the stud male. The LH peak did not occur until three hours post-coitum and on average lasted 5.7 hours (5/5 females). No LH surge occurred in 2/2 female ferrets in which only the copulatory behaviors prior to intromission were allowed to occur. This is the first systematic study to show that intromission per se, and not other courtship behaviors, is required for the ovulatory surge of LH in a reflex ovulator.

(Supported by U. S. Public Health Service grants HD-13634, MH-15761, and MH-00392.)

348.7 SEX DIFFERENCES IN THE RESPONSE OF LHRH NEURONS TO GONADECTOMY IN THE RAT: THREE-DIMENSIONAL COMPUTER ANALYSES. J.C. King, G. Kugel*, D. Zahniser*, K. Wooledge*, D. Damassa and B. Alexsavich*. Department of Anatomy and Cellular Biology, Tufts University School of Medicine and The Image Analysis Laboratory, Tufts-New England Medical Center, Boston, MA 02111.

Concentrations of plasma LH in rats increase following gonadectomy in a sex dependent manner. Whereas the rise in LH is immediate in males, it is delayed by 3-4 days in females. In this study we examined the response of preoptic/hypothalamic LHRH neurons to gonadectomy using the peroxidase-antiperoxidase unlabelled (PAP) immunocytochemical technique. It was the purpose of the study to ascertain: 1) the detectability of LHRH within neuronal perikarya at various times following gonadectomy in both male and female rats and 2) whether there was a difference in the distribution of LHRH at these times in male compared to female rats. The position of LHRH immunopositive neurons were mapped on sections from the Konig and Klippel atlas and digitized. The entire population of LHRH neurons was reconstructed in three dimensions using the MOVIE·BYU program. Images for groups of 4 male and 4 female rats sacrificed 1 day, 6 days and 3 weeks following gonadectomy were displayed on a high resolution TV system. Comparisons of these images indicate that the depletion of LHRH from neuronal perikarya is much more rapid in male than female rats. The detectability of LHRH within neurons is consistent with an early depletion from perikarya of the male due to augmented secretion followed by increased synthesis over a three-week period. The depletion of LHRH from perikarya of females appears to be delayed, possibly reflecting complex modulation of secretion by factors in addition to gonadal steroids. Furthermore, the distribution of LHRH neurons differed between the sexes suggesting a heterogenity of activity within this population. The antiserum used for these analyses, A-R419-obtained from A. Arimura, recognizes the decapeptide extended at both the N- and C-terminals as well as the decapeptide itself. These results may reflect the dynamics of intraneuronal processing of a putative precursor form of the active hormone following gonadectomy. This work was supported by the NIH 1KO4HDOO352, 5KO4HDOO485 and the NSF PCM8103243.

348.8 EFFECTS OF LHRH AGONISTS UPON LHRH NEURONS: IMPLICATIONS FOR FERTILITY REGULATION. M. M. Valenca*, C.A. Johnston, A. Negro-Vilar (SPON: K.-J. Chang). Rep. Neuroendo. Sect., Lab. Reprod. Devel. Tox., NIEHS, NIH, Res. Tri. Park, NC 27709.

Prolonged treatment with superactive agonists of LHRH suppress testicular or ovarian function in many species, including the human. Several reports have analyzed the actions of different LHRH agonists both at the pituitary and gonadal levels, but little is known about possible central actions that these peptides may have to modify reproductive functions. The present studies were designed to address that specific question, i.e., are there central effects of LHRH analogs following their systemic administration in regimens that result in testicular regression? Adult intact male rats (Sprague-Dawley, 70-90 days of age) were given either vehicle or an LHRH-agonist [D-Ala^6, Des-Gly^{10}] LHRH ethylamide, at a daily dose of 100 ng s.c. for 10 consecutive days. The medial preoptic (MPO), suprachiasmatic (SCN), medial septal (MS), rostral (AN_r) and caudal arcuate (AN_c) nuclei and the median eminence (ME) were microdissected, and LHRH was measured by radioimmunoassay. Treatment with the LHRH-A resulted in testicular atrophy, reduced sex accessory organ weights, and low testosterone levels in plasma and testicular interstitial fluid. After LHRH-A treatment AN_r and AN_c showed 2-4 fold increases in LHRH levels, whereas levels in ME were significantly decreased. To avoid changes in testosterone secretion, the LHRH-A was administered to animals orchidectomized immediately prior to the first LHRH-A injection. Control animals were orchidectomized and given the saline vehicle. Different groups of animals were sacrificed at 2, 7, and 10 days after the initiation of treatment. At all times after LHRH-A treatment, highly significant increments in LHRH levels were again seen in AN_r and AN_c. These changes were selective for the arcuate nucleus, since all other areas examined containing either LHRH cell bodies or nerve fibers and terminals showed no change in LHRH levels. The results indicate that: 1) the effects of the systemic administration of the LHRH-A upon brain LHRH neurons may reflect a direct central action of the peptide, since they were observed also in orchidectomized animals, which precluded any interaction with testicular products; and 2) the specific effects of LHRH-A upon the arcuate nucleus, an important area concerned with the regulation of gonadotropin secretion, suggest that the antigonadal activity of LHRH-analogs may involve a central (hypothalamic) action, in addition to the well known pituitary and testicular sites of action.

348.9 GABA REGULATION OF LUTEINIZING HORMONE SECRETION AND HYPOTHALAMIC CATECHOLAMINE ACTIVITY IN THE FEMALE RAT. B.A. Adler and W.R. Crowley. Dept. of Pharmacology, Univ. of Tennessee Center for Health Sciences, Memphis, TN 38163.

Recent evidence suggests that gamma-aminobutyric acid (GABA)-containing neurons may inhibit the release of luteinizing hormone (LH) under certain circumstances. The present experiments tested 1) whether GABA agonists block the LH surge induced in ovariectomized rats by estradiol benzoate (EB) followed by progesterone (P), and 2) whether these agents affect the depletion of hypothalamic catecholamines after synthesis inhibition, a measure of catecholaminergic activity. Ovariectomized rats received EB, followed 2 days later by P. Concomitant with P, rats received either saline, the GABA B agonist, baclofen, or the GABA agonist, muscimol. Other agonist-treated rats received a second injection 4 h later, or were additionally treated with the GABA A antagonist, bicuculline, or the putative GABA B antagonist, 5-aminovalerate. Additional experiments tested the effects of these agents on LH release in ovariectomized, hormonally untreated rats or in rats receiving exogenous LH-RH. The LH surge induced by EB plus P was blocked by administration of either baclofen or muscimol. Bicuculline or 5-aminovalerate were ineffective in preventing the effect of baclofen, but partially antagonized the effect of muscimol. Neither baclofen nor muscimol significantly altered LH release in ovariectomized rats or in rats receiving LH-RH. The EB plus P-induced LH surge is known to be dependent upon hypothalamic catecholamine activity. In other studies, EB plus P-treated rats were given saline, baclofen or muscimol with P and sacrificed 3 h later. One hour before decapitation, one half received the catecholamine synthesis inhibitor, alpha methyl tyrosine (AMT). Baclofen and muscimol significantly decreased the concentrations of norepinephrine and epinephrine in the medial preoptic and medial basal hypothalamic areas and prevented their decline after AMT, suggesting decreased catecholamine transmission. Dopamine was unaffected. These results suggest that an intrahypothalamic GABAergic system may depress LH release, perhaps in part by decreasing activity in hypothalamic noradrenergic and adrenergic systems that regulate LH-RH neurosecretion. This may occur at GABA B receptors located on catecholamine nerve terminals.

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348.10 STIMULATION OF LUTEINIZING HORMONE RELEASE BY QUINOLINIC ACID IN THE ADULT FEMALE RAT. M.D. Johnson, W.O. Whetsell, Jr., B.L. Carroll*, and W.R. Crowley.Dept. of Pharmacology, Univ. of Tennessee Center for Health Sciences, Memphis, TN 38163 and Dept. of Pathology, Vanderbilt Univ. Sch. of Med. Nashville, TN 37232.

The present studies examined whether the tryptophan metabolite, quinolinic acid (QUIN), a recently identified endogenous excitatory amino acid, or other agonists at excitatory amino acid receptor subtypes affect the release of luteinizing hormone (LH) in adult, ovariectomized, estrogen-primed female rats. Females were given 50 ug of estradiol benzoate, sc, 2 weeks after ovariectomy. Two days later, rats received intracisternal injections of either acidic saline vehicle, QUIN (250 or 500 nmol), N-methyl-DL-aspartate (NMA; 250 or 500 nmol); monosodium glutamate (Glu; 1.0 umol), or pyroglutamate (pGlu; 1.0 umol). Additional animals treated with QUIN or NMA received at the same time either 2-amino-7-phosphonoheptanoic acid (APH) or kynurenic acid (KYA), both antagonists at NMDA-preferring amino acid receptors, or glutamate diethyl ester (GDEE), an antagonist at quisqualate-preferring amino acid receptors. Animals were killed 5 min after drug treatment, and serum LH concentrations were determined by double antibody radioimmunoassay. Administration of QUIN or NMA resulted in a dose-dependent increase in serum LH levels. Coadministration of APH blocked these effects, and the QUIN stimulation of LH was also antagonized by KYA. Neither Glu nor pGlu increased LH release, and GDEE failed to prevent the elevation of LH by QUIN. In other studies, subcutaneous administration of NMA or QUIN failed to significantly stimulate LH release. Light microscopic evaluation of the hypothalamus showed no morphologic disturbances resulting from QUIN or NMA administration. These results suggest that QUIN, or other endogenous excitatory amino acids may stimulate LH release in adult female rats via an action at central NMDA-preferring receptors. In subsequent studies, depletion of brain catecholamines, achieved by pretreatment with a dopamine-B-hydroxylase inhibitor, did not prevent the increase of LH induced by intracisternal QUIN. However, administration of the serotonin synthesis inhibitor, p-chlorophenylalanine, or the serotonin antagonist, methysergide, attenuated the QUIN, but not NMA, stimulation of LH release. Thus, QUIN may increase LH release via an interaction with central serotonergic systems.

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348.11 INTRAGASTRIC CANNULATION IS A RELIABLE METHOD FOR ETHANOL ADMINISTRATION FOR NEUROENDOCRINE STUDIES. W. Les Dees* and Gerald P. Kozlowski. Department of Physiology, University of Texas Health Science Center at Dallas, Dallas, TX 75235

Stress has been shown to alter the release of LH, FSH and prolactin (Prl) in the rat (Ajika et al., Endo. 90:707,1972); thus, commonly used methods for short-term ethanol (ETOH) delivery such as i.p. or gastric gavage injections introduce the possibility that hormonal responses of individual animals could vary according to their ability to cope with the stress. Therefore, when using the rat to study the effects of ETOH on the hypothalamo-hypophysial-axis it would be important to utilize a method of ETOH delivery which is reliable, but would minimize stress. In this regard, 14 day ovariectomized rats weighing 300-350g were surgically implanted with a permanent intragastric cannula. ETOH was given either by an ETOH-saline solution, or via a liquid diet. Rats receiving the ETOH-saline solution (3.0g ETOH/Kg) were injected via the gastric cannula every 8 hr. for 3 consecutive days. Control animals received injections of saline only. Both groups received Lab Chow and water ad libitum. Each animal receiving the liquid diet regimen (Bio-Serve, Inc.) was provided with 40 ml of the ETOH or the isocaloric control diet ad libitum during the lights-off period, followed by 40 ml of the respective diet via the gastric cannula (4 injections of 10 ml each) equally divided over the lights-on period. Additional control animals were cannulated and maintained on Lab Chow and water, but were left untreated. Rats were decapitated 1 1/2 hr. after the last injection, serum collected, and blood ETOH levels determined by an enzymatic method (Sigma). Serum LH, FSH and Prl were analyzed by RIA. Results indicated that both groups of ETOH-treated rats showed significantly lower LH levels with significantly higher Prl levels when compared to control animals. Conversely, ETOH failed to alter FSH levels. No differences were detected between control groups. These results suggest that ETOH can differentially alter gonadotropin secretion, supporting the hypothesis that there may be a separate neural control mechanism governing FSH release. Also, this method of ETOH delivery minimizes possible stress induced changes in serum hormone levels, making it a reliable method for determining short-term effects of ETOH on the hypothalamo-hypophysial-axis. Supported by AA06014.

348.12 A SEQUENTIAL BLOOD SAMPLING SYSTEM FOR THE DETERMINATION OF PITUITARY HORMONE SECRETION PROFILES. D.I. Whitmoyer. Dept. of Anatomy and Lab. of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Studies of the correlations between central nervous system electrical activity and anterior pituitary hormone secretion require detailed timing information about events in both systems. Continuous monitoring of electrical activity is easily accomplished but no methods exist for performing a similar operation on the concentration of hormones circulating in blood. However, the time course of hormone release can be approximated by radioimmunoassay measurements on discrete blood samples. In the case of luteinizing hormone (LH), frequent sampling is required to insure that the sequence of hormone determinations adequately describes the underlying continuous process.

The system consists of 2 interconnected loops. The first one is a simple closed loop which uses a peristaltic pump to continuously circulate blood from and to the chronically-implanted unanesthetized rat via a double-lumen cannula inserted into the right atrium. Crosstalk between the input and output streams is minimized by the rapid blood flow in the atrium. Reconstituted whole blood is delivered into the return line on a sample-by-sample basis to compensate for the volume of blood removed from the loop. A positive-displacement metering pump removes a whole blood sample of known volume from the first loop and delivers it into the second loop. In this open loop, assay buffer is added and the bolus of sample plus buffer is pushed out through the exit line by a low-pressure stream of nitrogen gas. The line is connected to a fraction collector which advances in synchrony with the cycling of the sample metering pump. At the end of the trial, primary antiserum is added to the tubes and they are taken through a double-antibody radio-immunoassay procedure which is specialized for whole blood samples but uses standard NIADDK reagents for rat LH.

The current version of this automatic system allows inter-sample intervals ranging from many minutes down to about 20 seconds. Samples taken from long-term ovariectomized rats, at a rate of 1 per minute, clearly reflect the episodic or pulsatile nature of LH secretion known to occur in such animals. Typically the rapid rising phase is defined by 2 or 3 points and the ensuing exponential-like decay is delineated by 10 or more points.
(Supported by NIH grant NS01162.)

348.13 EFFECT OF ISOCARBOXAZID ON GROWTH HORMONE. V.S.Wahby*, E.L.Giller and K.Cohen*. Depts. of Psychiatry and Endocrinology, VA Medical Center and Yale University, West Haven, CT 06516

BACKGROUND: Tricyclic antidepressants can raise blood GH levels. It is not known whether monoamine oxidase (MAO) inhibitors e.g. isocarboxazid (Marplan), have the same effect.

METHODS: Ten male subjects ages 30-55 with major depressive disorder who have been on doses of isocarboxazid between 20-60 mg/day for at least three months were studied. All subjects were of normal weight, nondiabetic, non-alcoholic and with no evidence of other systemic disease or endocrinopathy. Liver and thyroid function tests were normal. The patients were known to be reliable and compliant in taking their medication. Their platelet MAO levels were 2.9-25.6 nmol/mg protein/hr. Three patients were simultaneously on diazepam 5-20 mg po/d. All other patients were on no medications other than isocarboxazid. Blood was drawn at 8:30 am before breakfast and after a normal overnight sleep, for a baseline GH level measured by RIA.

RESULTS: Two patients (20%) had a small but significant elevation in basal GH levels. In one patient this may be partially a diazepam effect. The rest of the patients (80%) had no significant GH elevations.

CONCLUSION: The MAO inhibitor isocarboxazid (Marplan), unlike tricyclics, may only have a minor effect of GH secretion in patients with MDD. An intriguing possibility is that a functional disturbance in central noradrenergic neurons in some MDD patients may have resulted in the blunting of an otherwise significant GH response to the drug. A similar finding with desipramine was reported by Laakmann. Further work is needed to verify this preliminary finding in larger samples of patients and healthy controls of both sexes stratified by age, body weight, MAO dose, clinical state at the time of testing and any other significant factors.

348.14 MULTIPLE INJECTIONS OF GROWTH HORMONE RELEASING FACTOR (GRF) DECREASE BASAL GROWTH HORMONE (GH) AND SOMATOMEDIN (Sm) CONCENTRATIONS IN SHEEP. M.A. Della-Fera, F.C. Buonomo* and C.A. Baile. Nutrition Chem. Div., Monsanto, St. Louis, MO 63137

GRF is a potent and specific releasor of GH in a variety of species. Previous results, however, show that the response to GRF declines with each subsequent treatment when multiple injections are given. Our objective was to determine the frequency that GRF could be administered to lambs without the development of refractoriness. Treatments were .016 nmol/Kg hpGRF(40) injected IV every 4, 8, or 24 hr for 3 days (N=12, 15 Kg b.w.). Plasma GH was measured (RIA) at -15, 0, 5, 15, 30 and 60 min (GRF given at 0). Sm was measured (RIA) at 0 and 120 min post injection. Decreased responsiveness to GRF developed even in the group injected once every 24 hrs. Peak plasma GH levels (+5 min) decreased from 105 ng/ml on day 1 to 35 and 19 ng/ml on days 2 and 3, respectively. Lambs injected every 8 hr showed no significant response to GRF by the second injection on the first day (peak after 1st injection:105ng/ml; peak after 2nd injection:29 ng/ml), and did not recover during the 3 day treatment period. Lambs injected every 4 hrs showed a similar decrease in response to GRF as those injected every 8 hrs. All three groups also showed a decrease in basal GH concentrations (preinjection) over the three day treatment period. In lambs injected every 24 hrs basal GH decreased from 23 (day 1) to 17 ng/ml (day 3; $p<.05$). In lambs injected every 4 or 8 hr GH decreased from 27 and 29 ng/ml (day 1) to 15 and 12 ng/ml (day 3; $p<.01$), respectively. Somatomedin also decreased by days 2 and 3 in lambs injected every 4 or 8 hrs with GRF (days 1, 2, 3, respectively: 4.6, 3.0, 2.4 IU/ml in lambs injected every 4 hr; 3.9, 2.5, 2.5 IU/ml in lambs injected every 8 hr. Values within a treatment differ between days, $p<.05$). IV bolus injections of GRF in young lambs, thus, cause a long-lasting and dramatic decrease in pituitary responsiveness to subsequent injections of GRF, and possibly to endogenous GRF, as evidenced by the decrease in basal plasma GH and Sm levels. The mechanisms involved in the decreased pituitary responsiveness may include increased somatostatin secretion, GRF receptor down-regulation, changes in post-receptor mechanisms involved in GH secretion, suppression of endogenous GRF secretion or decrease in GH synthesis.

348.15 A POSSIBLE ROLE FOR DIACYLGLYCEROL IN REGULATING GROWTH HORMONE RELEASE FROM ANTERIOR PITUITARY CELLS. A.M. Judd*, K. Koike*, T. Yasumoto* and R.M. MacLeod. Dept. of Internal Medicine, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

Growth hormone releasing factor increases pituitary growth hormone (GH) release and phosphatidylinositol (PI) metabolism. Diacylglycerol (DG), one of the metabolic products of phospholipase C (PL-C) hydrolysis of PI, is a potent stimulator of a Ca^{2+}- and phospholipid-dependent protein kinase (PKC). A study was performed to determine whether PKC is involved in mechanisms regulating GH release. Primary cultures of anterior pituitary cells were incubated for 15 minutes with synthetic DG (1-oleoly-2-acetyl-glycerol) or phorbol myristate acetate (PMA), a potent stimulator of PKC. DG increased the release of GH in a concentration-dependent manner (Control, 108 ± 10 ng GH; 50 ug/ml DG, 592 ± 65 ng GH; 500 ug/ml DG, 1180 ± 33 ng GH). Similar results were obtained with PMA (Control, 116 ± 62 ng GH; 100 nM PMA, 525 ± 50 ng GH; 1 uM PMA, 1096 ± 131 ng GH). In another study PL-C was added to pituitary cell cultures to liberate endogenous DG and it was found that PL-C increased GH in a dose-related manner over the range of 100 to 1000 mU/ml. In several tissues, the effects of DG on PMA were enhanced by sub-threshold concentrations of Ca^{2+} ionophore A23187. We found that 400 nM A23187, a dose that does not affect GH release by itself, enhanced (200%) GH secretion induced by either DG or PMA. Likewise 2 ng/ml maitotoxin, a Ca^{2+} channel activator which at this concentration has no effect on prolactin release, enhanced (200%) both DG- and PMA-induced prolactin release.

These data suggest that the hydrolysis of phospholipid to DG may activate PKC, that subsequently acts in concert with small changes in cytoplasmic Ca^{2+}, and thus may play a role in the regulation of GH release.

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348.16 ONTOGENY OF NORADRENERGIC REGULATION OF GROWTH HORMONE SECRETION IN RATS. L. A. Berg and C. M. Kuhn (Spon: T. Slotkin). Dept. of Pharmacology, Duke Univ., Med. Ctr., Durham, N. C. 27701.

In adult rats, the noradrenergic system plays a role in pulsatile growth hormone (GH) secretion. Blockade of noradrenergic receptors or disruption of norepinephrine synthesis prevents episodic bursts of GH release. Stimulation of α_2 adrenergic receptors is thought to mediate these effects. In addition, an α_1 mechanism which inhibits GH release has been postulated. The present studies demonstrate that immature rats possess an α_2 stimulatory mechanism which is active even in the absence of episodic surges of GH. An α_1 inhibitory mechanism may also exist in neonatal rats. We also offer evidence that opiates act through an α_2 stimulatory pathway to increase GH secretion in immature and adult rats.

In the first study, the effect of adrenergic agonists and antagonists was examined in developing rats. Dose response curves were obtained for a series of α_1 and α_2 agonist and antagonists. Ten day old rat pups were sacrificed after sc administration of drug and plasma samples were analyzed for GH by radioimmunoassay. Clonidine, an agonist specific for α_2 receptors at low doses, significantly increased GH levels at 50 µg/kg. A high (nonspecific) dose of clonidne (250 µg/kg) decreased GH. An intermediate dose of 150 µg/kg did not alter GH levels. The α_1 agonists, phenylephrine and methoxamine, suppressed GH at the highest dose analyzed (2.5 mg/kg). The α_1 antagonist prazosin, did not alter GH levels at any dose. In contrast, the α_2 antagonist yohimbine, at the highest dose of 2.5 mg/kg, significantly decreased GH.

Noradrenergic involvement in opiate induced GH secretion was examined in 10 day old and 31 day old rats. Yohimbine or prazosin was administered one hour before methadone and rats were sacrificed 30 minutes following methadone. Yohimbine significantly attenuated the GH increase induced by methadone, while prazosin did not. Prazosin or yohimbine alone did not alter basal GH levels at the doses used.

Our findings suggest that noradrenergic mechanisms participate in the regulation of GH secretion in neonatal rats as well as adult rats. A tonically-active α_2 system is demonstrable in neonates despite the absence of GH surges. The opiate response of GH appears to be mediated through this mechanism. Neural regulation of surging may develop as a separate pathway or become superimposed on the neonatal circuit.

348.17 GONADAL STEROID MODULATION OF GROWTH HORMONE-RELEASING FACTOR-STIMULATED GROWTH HORMONE SECRETION. G.F. McCormick*, W.J. Millard, T.M. Badger, and J.B. Martin. Depts. of Neurology and Gynecology, Mass. Gen. Hosp.,Vincent Res. Labs., Boston, MA 02114.

Hypothalamic growth hormone-releasing factor (GRF) is essential for spontaneous pulsatile growth hormone (GH) secretion. The pattern of pulsatile secretion is strikingly sexually dimorphic in the rat. We have investigated the influence of gonadal steroids upon GRF-stimulated GH secretion. In all experiments age-matched Sprague-Dawley rats were used. All studies were performed 6 to 8 weeks after castration. In the first experiment four groups were compared: Normal males, castrated (CAST) males, normal females, and ovariectomized (OVX) females (n=8, each group). Pairs of rats were anesthetized with pentobarbital, 35 mg/kg ip, to normalize plasma GH levels and permit insertion of cannulas into the right atrium. Blood samples were drawn at 15 minute intervals, beginning 45 minutes after PB administration. After three baseline samples, 10 ug/kg human pancreatic GRF 1-44 (GRF-44) was given iv. In all rats plasma GH values peaked within 5 to 10 minutes after GRF administration. The area under the GH peak was significantly greater in males than females. Castration decreased the response in males and ovariectomy increased the response in females. Pituitary content of GH correlated positively with the magnitude of GRF-stimulated GH secretion: normal males - 762.4 ± 33.2; CAST males - 566.1 ± 27.8; OVX females - 472.5 ± 51.6; and normal females - 341.1 ± 24.1 ug GH/mg soluble protein. In a second group of experiments, plasma levels of gonadal steroids were manipulated by subcutaneous implantation of steroid-filled silastic capsules. Four groups of rats were used: CAST males given testosterone (T), CAST males given estrogen (E), OVX females given T,and OVX females given E (n=8, each group). Silastic implants were relocated every 2 weeks for 8 weeks. GRF-44, 10 ug/kg iv, was given by the same protocol described above. Males and females treated with T had significantly greater peak areas than males and females treated with E. T treatment was associated with higher pituitary content of GH and faster growth rates. In conclusion, T increases and E decreases pituitary GH content and the magnitude of GRF-stimulated GH secretion.

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348.18 THIRD VENTRICULAR INJECTIONS OF NEUROPEPTIDE Y DECREASE LH AND GH SECRETION IN OVARIECTOMIZED RATS. J.K. McDonald, M.D. Lumpkin*, W.K. Samson* and S.M. McCann. (JKM) Dept. of Anat., Emory Univ. Sch. of Med., Atlanta, GA 30322 and Dept. of Physiol., U.T.H.S.C.D., Dallas, TX 75235.

In 1982, Neuropeptide Y (NPY) was isolated from porcine brain by Tatemoto and colleagues. NPY appears to be the endogenous peptide which accounts for descriptions of avian and bovine pancreatic polypeptide (APP & BPP) immunoreactivity in the brain (Card et al., 1983, Olschowka et al., 1981). We have reported that third ventricular (3V) injection of APP & BPP decrease plasma GH and LH in ovariectomized (OVX) rats (McDonald & Lumpkin, Endo Soc Abst 285, 1983). We report here the effects of 3V injection of NPY on plasma LH, GH and FSH in conscious OVX rats. NPY (5,2,0.5 and 0.02µg/2.0µl saline, Peninsula) or an equal volume of saline was injected via chronically implanted 3V cannulae and blood (0.6 ml) withdrawn through an indwelling jugular cannula immediately before (0) and 5,15,30,60 and 120 min after injection. NPY (5µg) reduced plasma LH at 15,30,60 and 120 min vs. pretreatment levels ($P<.05$, .005, .001, .001) and also vs. saline-injected controls at 30,60 and 120 min ($P<.025$, .005, .005). 2 and 0.5µg NPY produced similar effects, however, some recovery from inhibition was observed between 60 and 120 min. The 0.02µg dose caused a nonsignificant decline in LH at all times vs. controls. Rats receiving 5 or 2µg NPY displayed a sustained reduction in GH at several times vs. controls. Injection of saline or 5µg NPY reduced GH from 0 to 5 min and treatment with NPY prevented the subsequent rise in GH levels measured in controls. In another experiment, plasma GH in saline-injected rats did not decline to the same extent from 0 to 5 min, however, infusion of NPY (2µg) produced an immediate reduction in GH levels which remained below control values (60 min, $P<.025$) for the duration of the sampling period. 0.5 and 0.02µg NPY had no effect on GH, and NPY (2 and 0.02µg) had no effect on plasma FSH.

These results provide the first indication that NPY may be involved in the central regulation of LH and GH secretion. In view of the anatomical distribution of NPY in hypothalamic neurons and brainstem catecholaminergic afferents, NPY may affect LHRH, SRIF and GHRH neurons either directly or through neuromodulatory effects on catecholaminergic terminals involved in releasing hormone secretion. We have recently obtained data which indicate that NPY stimulates LH and GH secretion from perfused columns of anterior pituitary cells. (Supported by NIH HD-09988 and AM-10073).

348.19 STIMULATION OF GROWTH HORMONE SECRETION BY BETA-ADRENERGIC RECEPTOR ACTIVATION IN INTACT AND HYPOTHALAMUS-LESIONED RATS. R.J. Krieg, Jr., S.N. Perkins*, A.D. Rogol* and M.J. Cronin. Dept. Anatomy, Medical College Va., Richmond, VA 23298, and Depts. Physiol., Pediatrics and Pharmacol., Univ. Va. School of Medicine, Charlottesville, VA 22908.

Previous in vitro studies have shown that beta-adrenergic stimulation of perifused rat anterior pituitary cells results in growth hormone (GH) release (Neuroendocrinology 37:473, 1983). To determine whether this response exists in vivo, we investigated the effect of a beta-adrenergic agonist (isoproterenol) on GH release in adult male rats. After intra-atrial catheter implantations on Day 0, half the animals received lesions designed to isolate the pituitary gland from the basal hypothalamus. The lesions were made by lowering a triangular, 3mm Halasz knife to the base of the hypothalamus and rotating it several times; only rats in which significant portions of the basal hypothalamus were destroyed were included in the anaylsis. On Day 4, animals were anesthetized with pentobarbital (40mg/kg) to inhibit somatostatin release in the unlesioned rats, and sequential blood samples with saline replacement were taken at -10, 0, +2, +4, +6 and +10 min. Either saline, isoproterenol (200ug/kg), or human pancreatic GH releasing factor-40-OH (GRF, 400ng/rat) was infused immediately after the time 0 sample. Plasma hormones were measured by RIA. Whereas basal levels of prolactin remained unchanged in the lesioned rats, basal GH levels were significantly lower in lesioned versus unlesioned animals on Day 4 ($p=0.020$). Saline infusion did not alter GH secretion in either group. With one exception among 21 rats, isoproterenol induced an increase in circulating GH in both unlesioned ($p=0.014$) and hypothalamus-lesioned ($p=0.005$) animals (compared to preinfusion levels at time 0.) GH secretion was elevated at the earliest time point measured (+2 min) and decreased thereafter to reach control levels by the 10 min sample. GRF induced a 5-fold greater release of GH at +2 min than did isoproterenol. These results suggest that beta-adrenergic agonists can induce GH release in vivo, and that the hypothalamus may not be necessary for this effect. Our previous in vitro results demonstrated a direct and specific stimulatory action of isoproterenol and epinephrine on anterior pituitary GH secretion. The complimentary data from these in vivo and in vitro approaches reinforce our hypothesis that epinephrine may act as a GRF separate from the GRF peptide. (Supported by 5T32HL07284, RCDA NS00601, NS18409, AM32632)

348.20 PROKARYOTIC EXTRACYTOPLASMIC ADENYLATE CYCLASE EVOKES GROWTH HORMONE AND PROLACTIN RELEASE FROM NORMAL AND CLONAL PITUITARY CELLS. M.J. Cronin, A.D. Rogol*, J. Weiss*, A.A. Weiss* and E.L. Hewlett*. The University of Virginia School of Medicine, Charlottesville, Virginia, 22908.

We have been interested in the association of cAMP metabolism with growth hormone (GH) and prolactin (Prl) release from anterior pituitary (AP) cells. One criterion to prove a link between the cAMP-generating system and a biological event is that the response must occur when activated catalytic adenylate cyclase (AC) is added. Unfortunately, eukaryotic AC is labile when extracted and has not been functionally reconstituted into a cell. To circumvent this obstacle, we employed a novel and chemically robust extracytoplasmic AC (ECAC), synthesized by bacteria of the genus Bordetella (Hewlett, E.L., et al. PNAS 73:1926, 1976). We asked whether wild type Bordetella pertussis extracts, exhibiting marked ECAC enzymatic activity, affected rat AP cells in vitro. Cellular cAMP and hormone release were measured by RIA. In primary cultures of male AP cells and GH4C1 clonal cells, ECAC induced a dose-dependent increase in cAMP content (1.2 - 2.8 orders of magnitude) as well as GH and Prl release (0.3 - 2 orders of magnitude) at 1 and 2 hr. These changes were detectable at 5 min, the earliest time studied, and continued for at least 24 h. cAMP levels increased up to the highest concentration of ECAC imposed, while hormone release was maximal at lower levels of ECAC. These effects were apparently neither permanent nor toxic because cells responded appropriately to other secretagogues 1 day after a 2 h ECAC challenge and washout of the ECAC. In GH_3 cells, cholera toxin and forskolin effects on cAMP levels were additive with those of ECAC, indicating that ECAC does not activate native AC. With all of these agents, the cells' maximal secretory response was indistinguishable. Similar extracts from Bordetella parapertussis, which does not produce pertussis toxin but does synthesize ECAC, also increased cAMP levels and hormone release. In contrast, extracts of mutant strains of Bordetella pertussis, which do not produce ECAC or other virulence factors, did not increase cAMP accumulation. These studies demonstrate for the first time that endocrine cells can serve as targets for this novel bacterial AC and are consistant with the hypothesis that cAMP participates in the pathway of GH and Prl release. (Supported by RCDA 1K04NS00601, NS18409, AM32632, The DuPont, Rockefeller & Pratt Foundations, AI18000 & AM22125)

348.21 REPEATED ECS AND MORPHINE TOLERANCE: EFFECTS UPON NEUROENDOCRINE PARAMETERS. G.L. Belenky, J.R. Kenner*, L. Cardenales-Ortiz*, L. Pennington*, W. Gamble*, E. Mougey, J.L. Meyerhoff, and J.W. Holaday. Dept. of Med. Neurosci., Walter Reed Army Inst. of Research, Washington, DC 20307.

We have demonstrated that repeated electroconvulsive shock (ECS) sensitizes rats to the opioid effects of morphine, and that the induction of morphine tolerance sensitizes rats to the opioid-like effects of a single ECS. We have demonstrated that repeated ECS and the induction of morphine tolerance produce an increase in opioid receptors in whole rat brain. In the present study we compare the effects of repeated and single ECS and the induction of morphine tolerance in male Sprague-Dawley rats weighing between 250-300g upon neuroendocrine parameters - adrenal weight, plasma corticosterone, pituitary weight, and plasma prolactin. Repeated ECS (ECS given daily for nine days; sacrifice on day 10) increased adrenal weight over sham-repeated ECS control rats (RECS 63.8mg±2.7sem, Sham RECS 54.0mg±1.6sem; $t=2.68$, $df=78$, $p=.009$), and over rats receiving a single ECS on the first day (RECS 63.8mg±2.7sem, 1st day ECS 53.7mg±3.6sem; $t=2.78$, $df=78$, $p=.007$), and rats receiving a single ECS on the 9th day (RECS 63.8mg±2.7sem, 9th day ECS 55.9mg±2.4sem; $t=2.16$, $df=78$, $p=.033$). Induction of morphine tolerance (by pellet implantation) had no effect on adrenal weight. Repeated ECS raised plasma corticosterone over sham-repeated ECS control rats (RECS 11.0micrograms/100ml±2.0sem, Sham RECS 6.4micrograms/100ml ±0.9sem; $t=2.21$, $df=78$, $p=.03$). A single ECS on either the 1st or the 9th day, or the induction of morphine tolerance, were without effect on plasma corticosterone. Induction of morphine tolerance decreased anterior pitiuitary weight over placebo-pellet implanted control rats (Morphine pellet-implanted 1.44mg±.07sem, Placebo pellet-implanted 1.70mg ±.13sem; $t=2.07$, $df=78$, $p=.04$). Repeated or single ECS had no effect upon anterior pituitary weight. Neither repeated ECS nor induction of morphine tolerance had any effect upon combined intermediate and posterior pituitary weight. Induction of morphine tolerance lowered plasma prolactin in comparison to placebo-pellet implanted control rats (Morphine pellet-implanted 4.4ng/ml±.68sem, Placebo-pellet implanted 12.9ng/ml±2.3sem; $t=2.54$, $df=78$, $p=.01$). Neither repeated nor single ECS had any effect on plasma prolactin. Thus, while repeated ECS and induction of morphine tolerance share common effects upon opioid receptors, their effects upon neuroendocrine paramaters are disimilar.

348.22 ARE ACUTE OPIATE-INDUCED ALTERATIONS IN TSH SECRETION IN THE RAT SECONDARY TO CHANGES IN BODY TEMPERATURE? W.J. Litto and J. Rabii. Department of Biological Sciences and the Bureau of Biological Research, Rutgers University, Piscataway, N.J. 08854.

It has previously been shown that opiate agonists such as morphine sulfate (MS) acutely inhibit TSH secretion in adult male rats. Such studies, however, have focused on a single dose of MS or other agonists, and measurement of TSH at a single time point after administration. In order to characterize the acute TSH response to MS in more detail, we have generated a dose-response curve consisting of five doses of MS ranging from 0.1 to 20 mg/kg body weight (i.v.), and measurement of plasma TSH at 30 and 60 min. The low doses of MS produced significant inhibition of TSH with the magnitude of the inhibition decreasing as the amount of MS increased. Since MS has been shown to have a biphasic effect on body temperature in rats, with low doses producing hypothermia and higher doses causing hyperthermia, coupled with the fact that hypothermia stimulates TSH release, we hypothesized that the resulting hypothermia in animals receiving high doses of MS could be offsetting the inhibition of TSH release that was observed at low doses. Conversely, since it has been shown that hyperthermia will shut off TSH secretion, hyperthermia after low doses of MS could be causing inhibition of TSH release. In order to examine these possibilities, body temperature was monitored after MS administration. Thirty min after 1 mg/kg MS, TSH was significantly lower than preinjection levels, while body temperature was significantly elevated. Thirty min after 20 mg/kg MS, TSH levels were unchanged, while body temperature was significantly depressed. In order to determine whether changes in TSH induced by MS were preceded by alterations in body temperature, both variables were monitored at 5-min intervals for the first 15 min after drug administration. At 5 and 15 min after 1 mg/kg MS, TSH was significantly decreased, while the attendant hyperthermia was not manifested until 30 min. It can be concluded that the inhibition of TSH after 1 mg/kg MS is not caused by an increase in body temperature. The degree to which hyperthermia after low dose MS contributes to the inhibition of TSH release, however, remains to be determined. (Supported in part by NIH Grant DA02227 and grants from Charles and Johanna Busch Memorial Fund.)

348.23 FUNCTIONAL COUPLING OF AN OPIATE-SEROTONERGIC MECHANISM IN THE MATURATION OF THE PROLACTIN, BUT NOT THE PRO-OPIOMELANOCORTIN RESPONSE TO ETHER STRESS AND MORPHINE. C.A. Johnston and A. Negro-Vilar. Reprod. Neuroendo. Sect., Lab. Reprod. Develop. Tox., NIEHS, NIH, Res. Tri. Park, NC 27709

Our laboratory has shown that the maturation of the prolactin (PRL) response to ether stress, which is present in prepubertal (36 day old) and adult rats but not in infantile (13 day old) rats, is associated with an enhanced serotonin (5-HT) metabolism in the mediobasal hypothalamus (MBH). On the other hand, morphine can enhance PRL release and increase 5-HT metabolism in the MBH at all stages of development. This study analyzed the degree to which endogenous opiate and 5-HT systems participate in the regulation of the neuroendocrine response to ether stress. Because norepinephrine (NE) metabolism is increased following either ether stress or morphine, we evaluated the possible role of this NE activation in the neuroendocrine response to ether stress. Infantile and prepubertal male rats were treated as follows: a) control; b) naloxone; c) ether stress (3 min); d) naloxone followed by ether stress; e) morphine; f) naloxone followed by morphine. 5-HT and NE metabolism were evaluated using HPLC with electrochemical detection. Plasma PRL, ACTH, and β-endorphin (β-end) were measured by RIA. Morphine and ether stress both increased 5-HT and NE metabolism in the MBH and plasma PRL, ACTH, and β-end in prepubertal rats. Similar results were observed in infantile rats with the important exception that ether stress did not alter plasma PRL or 5-HT metabolism in the MBH. Naloxone alone did not alter 5-HT and NE metabolism or plasma levels of PRL, ACTH, or β-end but prevented every morphine-induced alteration in both infantile and prepubertal animals. Furthermore, naloxone prevented both the increase in MBH 5-HT metabolism and in plasma PRL caused by ether stress in prepubertal animals. However, naloxone did not prevent the ether stress-induced increases in NE metabolism or pro-opiomelanocortin-derived peptides (POMC's) in either age group. The results demonstrate that A) an endogenous opiate system is involved in the stress-induced release of PRL but not POMC's; B) both the morphine effect and the endogenous opiate effect during ether stress on PRL secretion require a mandatory increase in 5-HT metabolism in the MBH; C) the coupling of the endogenous opiatergic and 5-HT neuronal systems occurs sometime between day 13 and day 36 postnatally; D) the ether stress-induced changes in NE metabolism may be related to the release of POMC's and do not require an endogenous opiate system.

348.24 REGULATION OF HYPOTHALAMIC BETA-ENDORPHIN RELEASE IN THE RAT. J.I. Koenig, H.Y. Meltzer and G.A. Gudelsky. Dept. of Psychiatry, Univ. of Chicago and Ill. State Psychiatric Inst., Chicago, IL. 60637.

Immunocytochemical studies in the rat have revealed that beta-endorphin neuronal perikaryon reside within the mediobasal hypothalamus and one of the projections of these neurons is to the median eminence. Previous studies have demonstrated that beta-endorphin is present in hypophyseal portal blood. However, only limited data are available on the factors which influence the release of beta-endorphin into the portal vessels. The current studies were undertaken to elucidate the factors controlling beta-endorphin release. Hypophyseal portal blood was collected from urethane-anesthetized male rats. The blood samples were collected on ice in plastic tubes containing trasylol. Beta-endorphin levels were determined by RIA using an antiserum directed against human beta-endorphin which cross-reacts completely with rat beta-endorphin. This antiserum has limited cross-reactivity with beta-LPH and does not recognize met- or leu-enkephalin or alpha- or gamma-endorphin. Serial dilutions of rat pituitary stalk plasma were parallel to the beta-endorphin standard curve, confirming that beta-endorphin and not cross-reacting materials were being measured. Beta-endorphin concentrations in pituitary stalk plasma were 2.58 ± 0.77 ng/ml; systemic levels of beta-endorphin in the same animals were 0.47 ± 0.07 ng/ml. These systemic levels of beta-endorphin were significantly greater than the levels found in unanesthetized, decapitated rats (0.11 ± 0.02 ng/ml). Stimulation of the opiate receptors by morphine has been reported to elevate systemic beta-endorphin levels. In contrast, morphine infusion (3 mg/kg,i.v.) resulted in a 62% suppression of beta-endorphin levels in portal plasma. In addition, capsaicin has recently been shown to decrease hypothalamic beta-endorphin content by an unknown mechanism. The intraventricular administration of capsaicin (10 ug) on days 1 and 3 resulted in a decrease in stalk plasma concentration of beta-endorphin to 0.83 ± 0.11 ng/ml ($p < 0.025$) on day 4. Systemic levels of beta-endorphin, however, were unaltered by capsaicin treatment. These studies suggest that hypothalamic beta-endorphin is released into the hypophyseal portal circulation, and the mechanisms regulating the release of hypothalamic beta-endorphin differ from those which regulate beta-endorphin release from the pituitary gland.